

## Presentations and Posters of Interest

### Society for Neuroscience Meeting (2015)

#### 34.01/A100. *Estradiol rapidly attenuates ORL-1 receptor-mediated inhibition of proopiomelanocortin neurons via Gq-coupled, membrane-initiated signaling*

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Ovarian estrogens act through multiple receptor signaling mechanisms that converge on hypothalamic arcuate nucleus (ARH) proopiomelanocortin (POMC) neurons. A subpopulation of these neurons project to the medial preoptic nucleus (MPN) to regulate lordosis. Orphanin FQ/nociception (OFQ/N) via its opioid-like receptor (ORL-1) regulates lordosis through direct actions on these MPN-projecting POMC neurons. Based on an ever-burgeoning precedence for fast steroid actions, we explored whether estradiol excites ARH POMC neurons by rapidly attenuating inhibitory ORL-1 signaling in these cells. Experiments were carried out in hypothalamic slices prepared from ovariectomized female rats injected one-week prior with the retrograde tracer Fluorogold into the MPN. During electrophysiologic recordings, cells were held at or near -60 mV. Post-hoc identification of neuronal phenotype was determined via immunohistofluorescence. In vehicle-treated slices OFQ/N caused a robust outward current/hyperpolarization via activation of GIRK channels. This OFQ/N-induced outward current was attenuated by 17- $\beta$  estradiol (E2, 100nM). The 17 $\alpha$  enantiomer of E2 had no effect. The OFQ/N-induced response was also attenuated by an equimolar concentration of E2 conjugated to BSA. In addition, the ability of E2 to diminish OFQ/N responsiveness was blocked by the co-administration of the estrogen receptor (ER) antagonist ICI 162,780 (1 $\mu$ M). The attenuating effect of E2 was mimicked by the membrane ER (mER) ligand STX (10nM) and the ER $\alpha$  agonist PPT (1 $\mu$ M), but not the GPR30 agonist G1 (3 $\mu$ M) or the ER $\beta$  agonist DPN (3 $\mu$ M). Moreover, the phospholipase C (PLC) inhibitor U73122 (20 $\mu$ M) restored the OFQ/N-induced outward current in the presence of E2, whereas the inactive analog U73343 (20 $\mu$ M) was without effect. Finally, the protein kinase C (PKC) inhibitor NPC 15437 (30 $\mu$ M) abrogated the estrogenic impairment of the OFQ/N-induced outward current, whereas the PKC activator PDBu (1 $\mu$ M) per se attenuated the OFQ/N response. These collective actions were observed in a substantial number of MPN-projecting ARH neurons positive for various markers of POMC neurons. The results reveal an ORL-1 receptor mediated inhibition of POMC neurons that is negatively modulated by estradiol. The estrogenic attenuation is stereoselective; membrane delimited, mediated via the Gq-coupled mER and ER $\alpha$  activation, and involves signaling through PLC and PKC. This disinhibition of POMC neurons is critical for the subsequent expression of sexual behavior in the female.

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34.04/A103. ***Synapse-specific persistent activation of VTA kappa opioid receptors following acute stress***

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Stressful experiences drive many adaptive and maladaptive behaviors, and even acute stressors can have lasting behavioral consequences. Emerging evidence shows that dopaminergic neurons in the ventral tegmental area (VTA) are an important locus in stress. We previously identified a long-term potentiation of GABAergic synapses onto these neurons (LTPGABA) that is blocked by acute stress (Graziane et al, Neuron, 2013). Administration of a kappa opioid receptor (KOR) antagonist (norBNI) in vivo prevents the block of LTPGABA. Intra-VTA injection of the KOR antagonist also prevents reinstatement of cocaine seeking by acute stress, suggesting that KOR-mediated regulation of VTA inhibitory plasticity may play a role in stress-induced drug seeking. Our recent work shows that a single five minute cold water swim stress blocks LTPGABA for at least five days. Surprisingly, blocking KORs with norBNI even well after stress restores LTPGABA, and cocaine self-administration is prevented even when norBNI is administered after stress (Polter et al, Biological Psychiatry, 2014). Here we show that the long-lasting block of LTPGABA by stress is due to changes in the KOR itself. While bath application of an inverse agonist (norBNI, 10 nM) rescues LTPGABA in slices from stressed animals, bath application of a neutral antagonist (6--naltrexol, 10 μM) does not (LTP magnitude: norBNI after stress=144± 18% of baseline, 6--naltrexol after stress=99±8% of baseline; p<0.05). These results suggest that LTPGABA is blocked by constitutive activation of KORs rather than by persistently elevated dynorphin, which would be blocked by both drugs. Transient activation of KORs was sufficient to induce a lasting blockade of LTPGABA, as a KOR agonist (U50488, 5 mg/kg) blocked LTPGABA for 5 days (LTP: saline=140±10% of baseline, 1 day post U50488=108±5% of baseline, 5 days post U50488=99±9% of baseline). The activation of KORs by stress is synapse specific, as bath application of norBNI did not potentiate excitatory synapses on either dopaminergic (IPSC amplitude after norBNI: control=92±6% of baseline, FSS=94±2% of baseline) or GABAergic VTA neurons (IPSC amplitude after norBNI: control=109±4% of baseline, FSS=112±3% of baseline). Our results show that a single exposure to acute stress or KOR activation both cause long-lasting changes in activity of KORs specifically at GABAergic synapses in the VTA.

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34.05/A104. ***Variable sensitivity to morphine mediated Ferritin Heavy Chain upregulation in cortical neuronal subpopulations***

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Current HIV therapies have dramatically improved patients' disease progression and quality of life, but HIV-associated neurocognitive disorder remains prevalent and challenging problem to address in the clinic. Many HIV+ patients also abuse drugs, with opioid use being particularly common as sharing needles represents an important avenue of infection. These patients can show accelerated progression of cognitive impairment, the mechanism of which is currently under investigation. Our lab has shown that morphine and other mu-opioids upregulate Ferritin Heavy Chain (FHC) in CNS neurons, which is associated with reduction of downstream signals of the homeostatic chemokine receptor CXCR4 via its natural ligand CXCL12. This results in a host of adverse effects including reduced dendritic spine density and is correlated with enhanced cognitive decline in humans and animal models of HAND. Our experiments aim to gain insights into this novel mechanism of mu-opioid mediated CXCR4 regulation, and thereby potential future targets for novel HAND therapies. Morphine upregulates FHC in cortical neurons in a mu-opioid/g-protein dependent manner, while astrocytes seem to be unaffected in vitro even though they express the mu-opioid receptor. This suggests exclusivity for particular CNS cell types in their ability to upregulate FHC via opioids. FHC upregulation specifically occurs in the cytoplasm of neuronal cells as demonstrated by fractionation and confocal imaging studies, which allows it to potentially interact with CXCR4 on the cell membrane. Preliminary imaging studies show that certain neurons are more susceptible to FHC upregulation than others, and that GABA transporter-1 expressing neurons represent one of these susceptible populations. Current work is focused on exploring FHC expression after morphine treatment in inhibitory and excitatory neuronal subpopulations both in vitro and in vivo. Pilot studies suggest variability amongst neuronal subpopulations in that calretenin expressing interneurons do not upregulate FHC after morphine, but interestingly have higher basal levels of FHC compared to calretenin negative cortical neurons. Future studies will characterize additional neuronal populations. These experiments suggest that mu-opioid usage may cause specific deficits in inhibitory neuronal circuits via FHC upregulation and subsequent CXCR4 blockade, which may induce or sustain particular features of HAND such as excitotoxicity, or other neurochemical adaptations leading to cognitive impairment.

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34.14/B5. ***Localization of AGRP immunoreactivity in the mouse hippocampus that expresses eGFP-tagged GHSR1a***

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The ghrelin receptor (GHSR1a) is expressed highly in the hippocampus (Mani et al., 2014) and contributes to learning and memory (Diano et al., 2006). However, in spite of intense investigation with immunohistochemical techniques and transgenic eGFP expression, what types of hippocampal neurons express GHSR1a is not well understood. Available evidence suggests hippocampal principal neurons (glutamatergic pyramidal cells and dentate granule cells) are likely the target of ghrelin (Diano et al., 2006; Cuellar and Isokawa, 2011). However, in the hypothalamus, GHSR1a is specifically localized in AgRP/NPY/GABA-containing neurons (Wang et al., 2013). Since AgRP, NPY, and GABA are all well-

acknowledged neuropeptides and neurotransmitters in the hippocampus, this evidence suggests the possibility that a subset of hippocampal GABAergic interneurons that co-express AgRP and/or NPY could be the target of ghrelin. In the present study, localization of NPY and AgRP are assayed immunohistochemically in the brain of transgenic mouse strain where GHSR1a-expressing cells are reported with eGFP. Identification of GABAergic neurons are accomplished by the immunohistochemical detection of GAD, an enzyme to synthesize GABA. Cardially-perfused brains are cryo-sectioned into 50 micrometer thick. Brain sections that contained the hippocampus and the hypothalamus (as a positive control) are collected and processed for immunohistochemistry. NPY, AgRP, GAD, and eGFP signals are visualized using a confocal microscope and the results are quantified for analysis. We observed AGRP immunoreactivity at cell soma and processes in the dentate gyrus where eGFP signals for GHSR1a were also detected. AGRP immunoreactivity was particularly dense at the inner (hilar) edge of the dentate granule cell layer, supporting our hypothesis that AGRP neurons could be the target of ghrelin in the hippocampus. We propose a novel cellular mechanism for hippocampal learning and memory that involves AGRP/GHSR1a interactions.

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50.04/F19. ***Inactivation of CeA neuronal ensembles prevents alcohol drinking in dependent and non dependent rats***

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Withdrawal from alcohol is associated with the recruitment of neurons in the central nucleus of amygdala (CeA) in rats binge-drinking on alcohol (non-dependent) as well as in dependent rats. However, whether the recruitment of this withdrawal neuronal ensemble in the CeA is causally related to excessive drinking or represents a consequence of the excessive drinking remains to be demonstrated. Here we tested the hypothesis that inactivation of the withdrawal neuronal ensemble in the CeA is responsible for the excessive alcohol drinking observed in non-dependent binge drinking rats and in dependent rats. In order to test our hypothesis we investigated the effect of the inactivation of neuronal ensembles in the CeA using a pharmacogenetic approach (Daun02 inactivation method in Fos-Lac Z transgenic rats). One group of rats was made dependent using chronic, intermittent exposure to alcohol vapor and tested for alcohol drinking in 3 minutes session 6-8 hours into withdrawal. In a second group of animals (non-dependent binge-drinker) we tested the effect of Daun02 on ethanol intake using chronic intermittent access to two-bottle choice. We found that inactivation of neuronal ensembles by injection of Daun02 in the CeA significantly decreased alcohol drinking in both groups with the only difference that in non-dependent animals the decreased ethanol intake was transient the day of the injection and returned to normal the day after the injection, while in the dependent animals inactivation of the withdrawal neuronal ensemble produced a long-term decrease in alcohol drinking that lasted at least 2 weeks. We also found a significant reduction of the somatic withdrawal signs in the dependent animals injected with Daun02 in the CeA. These results demonstrate that the recruitment of

neuronal ensemble in the CeA during alcohol withdrawal is causally related to the excessive alcohol drinking observed in alcohol dependent rats but that this withdrawal neuronal ensemble only partially contributes to alcohol drinking in non-dependent rats.

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50.06/F21. ***Evaluating the reward- enhancing effects of nicotine on ethanol self-administration in male and female rats***

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Nicotine and alcohol dependence disorders are highly correlated: up to 80% of alcohol-dependent persons in the US smoke regularly, and risk of alcohol-dependence is four times higher among people who are nicotine-dependent. Additionally, there are significant differences in the prevalence and nature of nicotine- and alcohol-dependence between the sexes. Women, on average, are less likely to attempt to quit smoking and more likely to relapse after quitting. Additionally, women show greater sensitivity to the intoxicating effects of alcohol, slower metabolism of alcohol, and greater risk for adverse health consequences alcohol use. However, binge drinking and alcohol-dependence is more common among men than women. Understanding the behavioral mechanisms that underlie alcohol- and nicotine-dependence and their high comorbidity will require consideration of the multifaceted causes that include an individual's sex. There is mounting evidence that the reward-enhancing properties of nicotine synergistically enhances behavior directed at obtaining other rewards, and this reward-enhancement effect holds promise in deciphering the mechanisms that drive nicotine dependence. That is, nicotine administration that occurs in the same context as the reception of other rewarding stimuli may enhance their rewarding properties, and this enhancement may drive motivation to abuse nicotine. However, no published work to date has investigated the role that reward-enhancement may play in the comorbidity between nicotine- and alcohol-abuse. The present work investigated the role that reward-enhancement by nicotine plays in the comorbidity of nicotine- and alcohol-abuse using an operant ethanol self-administration procedure in male and female rats. Rats were trained to self-administer 15% ethanol using a sucrose fading procedure. Nicotine or saline was injected 10 min preceding 45 min ethanol self-administration sessions. Over blocks of sessions, the unit cost per gram ethanol was systematically examined across two separate reinforcer demand assessment phases: fixed-ratio schedule escalation and ethanol concentration reduction. A behavioral economic, reinforcer demand model was applied to the data from both unit cost manipulation phases and the effects of nicotine on ethanol reinforcement value were compared between the sexes and between unit cost manipulation procedures.

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50.20/F35. ***Interactive effects of ethanol and HIV-1 viral proteins on novelty-seeking behaviors***

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Novelty-seeking behavior is related to the reward system in the brain and could predict the potential for addiction. Alcohol use is prevalent in HIV-1 infected patients and adversely affects anti-retroviral medication. The difference in vulnerability to alcohol addiction between HIV-1 infected and non-infected populations has not been fully investigated. This study was designed to determine whether HIV-1 viral proteins alter the effects of ethanol on novelty-seeking behaviors in the HIV-1 transgenic (HIV-1Tg) rat. The hole board and open field tests were used to first compare the baseline (pre-session ethanol) novelty-seeking behaviors in HIV-1Tg and F344 control rats. Rats then received single daily intra-peritoneal injection of ethanol (1 g/kg) or saline for 13 d. The rats were then re-exposed to the hole board and open field test on the Days 10 and 11 (post-session ethanol trials) to determine the combined effects of HIV-1 viral proteins and chronic ethanol exposure on novelty-seeking behaviors. There was significant strain difference in baseline novelty-seeking behaviors; HIV-1Tg rats exhibited both higher head dip scores in the hole board test ( $p = 0.001$ ) and center entry scores ( $p = 0.01$ ) in the open field test compared to the F344 control rats. In the post-session ethanol trials, novelty-seeking behaviors were increased in the ethanol-treated groups, but decreased in the saline control groups in both the hole board ( $p = 0.001$ ) and open field ( $p = 0.004$ ) tests. Ethanol-treated HIV-1Tg rats had higher head dip ( $p < 0.01$ ) and center entry ( $p < 0.05$ ) scores compared to the ethanol-treated F344 rats. There was a significant difference in head dip ( $p = 0.03$ ) and center entry ( $p = 0.02$ ) scores in pre- and post-ethanol treatment sessions, respectively, in the HIV-1Tg rats. Our results indicate that HIV-1 viral proteins alter novelty-seeking behaviors, induce neuronal adaptations in the mesolimbic reward pathway, and enhance the effects of ethanol on novelty-seeking behaviors. Using the novelty-seeking trait as a correlative behavioral marker of alcohol dependence could have important implications for the development of new psychopharmacological treatments for alcohol-dependent HIV-1 infected patients.

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51.01/F37. ***Cocaine-induced alterations in structural plasticity in the dmPFC of rats during early withdrawal***

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Relapse to drug-seeking is a major clinical obstacle in treating addiction. Numerous studies have shown that dysregulation of the dorsomedial prefrontal cortex (dmPFC) to nucleus accumbens core (NAc core) glutamatergic projection precipitates relapse in rodent models. Altered synaptic plasticity and dendritic spine morphology and density (structural plasticity) within corticolimbic circuitry are associated with different phases of withdrawal and relapse. Previously, our lab has shown that cocaine self-administration (SA) in rats results in depression of ERK, CREB, and NMDA receptor subunits, GluN2A and GluN2B, phosphorylation. These signaling alterations are associated with parallel increase in striatal-enriched tyrosine phosphatase (STEP) activity in the dmPFC two hours after the final of 1 short access SA sessions (early withdrawal), suggesting decreased synaptic activity. A similar decrease in ERK

and CREB phosphorylation, as well as GluN2A/B expression, is associated with decreased apical dendritic complexity in stress and depression models. In light of these observations, the current study investigated whether cocaine SA decreases apical spine head diameter (dH) and density in the dmPFC during early withdrawal. Fifteen male Sprague Dawley rats self-administered cocaine or were exposed to non-contingent yoked saline infusions for 14 days, and were transcardially perfused two hours after the final session. Coronal sections containing the dmPFC were labeled with the lipophilic dye Dil, and apical dendrites were acquired with confocal microscopy, and analyzed with Imaris 3D image analysis software. Results indicate that cocaine SA decreases layer II/III apical spine density as well as layer V apical spine dH ( $p < 0.05$ ). The decreased layer II/III apical spine density is specific to loss of thin and mushroom type spines. Ongoing experiments are delineating whether these alterations are occurring in dmPFC neurons that project to the NAc core using retrogradely transported fluorescent microspheres injected into the NAc core. We predict this alteration in structural plasticity plays a role in decreased synaptic activity during early withdrawal. This alteration may be facilitating relapse following abstinence because a single BDNF microinfusion immediately after SA suppresses relapse by reversing a cocaine-induced depression of phosphoproteins in the dmPFC during early withdrawal. Thus, investigation as to whether a BDNF microinfusion, or STEP inhibition, will reverse these cocaine-induced alterations in structural plasticity is a future direction.

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51.02/F38. ***Alterations in dmPFC neuronal activity during and immediately after cocaine self-administration***

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Previous work from our laboratory has identified down-regulation of activity-related phospho-proteins (p-GluN2A/B, pERK and pCREB) in the dorsomedial PFC (dmPFC) during early withdrawal from cocaine self-administration (SA) that appears to be crucial for relapse. Reversing these changes with single infusion of brain-derived neurotrophic factor (BDNF) into the dmPFC immediately after the last cocaine SA session suppresses subsequent cue- and cocaine-induced drug-seeking in a TrkB- and ERK-dependent manner, and normalizes cocaine-induced dysregulation of glutamate levels in the nucleus accumbens (NAc) that are associated with relapse. Given the profound therapeutic potential of this understudied time point, the current study aims to explore direct measurements of neuronal activity in the dmPFC during SA and early withdrawal through the use of in vivo single-unit electrophysiological recordings in awake, behaving rats. Male Sprague Dawley rats were implanted with an intra-jugular catheter and a drivable 16-wire electrode bundle into the dmPFC and allowed to recover for 5 days. Rats were habituated to the SA chamber for 1 day before taking a 4 hr and 50 min baseline recording to assess the activity of dmPFC neurons in the absence of any stimulation. Rats were then trained to self-administer cocaine on an FR1 schedule, with electrophysiological recordings taken on Days 1-2 (Early SA) and Days 11-12 (Late SA). Neural activity was continually recorded during 2 min baseline, during the 1 hr cocaine SA period, and during the 2.5 hr post-cocaine SA period. Preliminary data suggest that cocaine

SA in cocaine-experienced (but not naïve) animals suppresses neuronal firing in the dmPFC when compared to the 20 min baseline. Interestingly, after the levers are withdrawn and cocaine access is taken away (indicating the beginning of early withdrawal), neuronal activity increases dramatically and is sustained at a higher rate of firing through the end of the session. This increase in neuronal firing during early withdrawal may paradoxically explain the decreases in NMDA receptor-related phospho-proteins that are observed 2 hr after the final SA session. It is likely that increases in neuronal activity following cocaine-induced suppression are robust enough to induce calcineurin-dependent phosphatases that dephosphorylate GluN2A/B receptor subtypes and ERK. These data show promise in expanding our understanding of the mechanisms involved during cocaine SA and in early withdrawal. Supported by DA033479

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51.03/F39. ***Inhibition of Src family kinases prevents the suppressive effect of BDNF on cocaine-seeking***

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Relapse to drug seeking remains a major obstacle in the treatment of cocaine addiction in human addicts. Animal models of relapse have demonstrated that neuroadaptations in reward circuits following cocaine self-administration underlie reinstatement to drug seeking. Specifically, dysregulation of the pathway from the prefrontal cortex (PFC) to the nucleus accumbens (NAc) is implicated in reinstatement. Brain-derived neurotrophic factor (BDNF) is synthesized in PFC pyramidal neurons and anterogradely transported to the NAc where it is the primary source of BDNF. Our lab has shown that a single BDNF infusion into the prelimbic cortex following a final cocaine self-administration session results in attenuation of reinstatement to cocaine-seeking. Inhibiting BDNF's receptor, TrkB, ERK/MAP kinase activation, or AMPA/NMDA receptors can block this attenuating effect. These results imply that the interaction between glutamate-mediated synaptic activity and TrkB signaling is imperative to BDNF's suppressive effect on drug-seeking. Src family kinases (SFKs) are involved in both NMDA/AMPA-mediated activation of TrkB and TrkB-mediated phosphorylation of NMDA receptors. Thus SFKs serve as likely link between these two signaling systems. We hypothesized that infusion of the SFK inhibitor, PP2, into the prelimbic cortex prior to BDNF infusion immediately after the end of the last cocaine self-administration session will block BDNF's attenuation of both context- and cue-induced reinstatement in rats. PP2 blocked BDNF's suppressive effect on context-induced relapse after one week of abstinence and cue-induced reinstatement after extinction. Because cocaine induces a dephosphorylation of GluN2A and GluN2B receptors and BDNF reverses this action, PP2 is likely blocking this reversal. Analysis of phospho-GluN2A/B, phospho-ERK, and activated SF levels performed on the automated immunoassay system WESTM (Protein Simple, BioTechne) will be presented to determine if PP2's blocking action occurs from dysregulation of TrkB-mediated NMDA receptor activation.

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51.04/F40. ***Involvement of CaMKII within the prefrontal cortex and the nucleus accumbens in the effects of Taar1 agonist on reinstatement of cocaine seeking in rats***

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The trace amine-associated receptor (TAAR1), novel G protein coupled receptor, has proven to play a crucial role in modulating the dopaminergic system in brain. Our recent study demonstrated that systemic administration of TAAR1 agonist decreased cue- or drug-induced reinstatement of cocaine seeking in rats. However, the neuronal mechanism underlying the role of TAAR1 in cocaine addiction remains unknown. Here, we examined the effect of selective TAAR1 agonist RO5166017 on cocaine seeking behavior in rats, and investigated the underlying mechanism. Rats were tested drug-induced reinstatement one day after extinction of cocaine self-administration. Immediately after cocaine reinstatement test, rats were decapitated and tissues of two critical brain regions in drug addiction, the prefrontal cortex (PFC) and the nucleus accumbens (NAc), were harvested for examining the alterations of related molecules. The results showed that pretreatment of RO5166017 (10 mg/kg, i.p.) reduced cocaine priming-induced reinstatement of cocaine seeking. After reinstatement, the levels of phosphorylated Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (pCaMKII) and phosphorylated extracellular signal-regulated kinases 1/2 (pERK1/2) in both brain regions were elevated. RO5166017 prevented cocaine priming-induced increase in pCaMKII in both the PFC and the NAc, but it did not affect the level of pERK1/2. Furthermore, bilateral microinjection of RO5166017 (5 µg/0.5 µl/side) into the prelimbic cortex of PFC and NAc shell both inhibited cue- and drug-induced reinstatement of cocaine seeking. These results suggested that CaMKII-dependent signaling pathway within the PFC and the NAc may mediate the role of TAAR1 in reinstatement of cocaine seeking.

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51.05/F41. ***nNOS-expressing interneurons: A master switch for nucleus accumbens plasticity underlying cocaine relapse***

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Chronic cocaine exposure produces neuroplasticity within the nucleus accumbens core (NAcore) that leads to increased vulnerability to relapse, even after protracted abstinence. Relapse is associated with a transient synaptic potentiation of corticostriatal synapses in the nucleus accumbens core, and the magnitude of this potentiation is correlated with the intensity of relapse behavior. Matrix metalloproteinases (MMPs) are Zn<sup>2+</sup>-dependent endopeptidases that degrade the extracellular matrix to promote synaptic plasticity, and recent work from our lab has shown that upregulated MMP activity is required for synaptic plasticity accompanying cocaine addiction. One mechanism of MMP activation is through S-nitrosylation via nitric oxide (NO). NO is synthesized in a small subset of GABAergic

interneurons within the accumbens (~1%), and these neurons are characterized by the expression of neuronal nitric oxide synthase (nNOS). We hypothesized that nNOS activity and MMP S-nitrosylation is both necessary and sufficient to drive cue-induced reinstatement of cocaine seeking. In order to test this, we used NOS1-Cre transgenic mice for selective targeting of nNOS-expressing interneurons. By stimulating Ca<sup>2+</sup> signaling via Cre-dependent Gq-DREADD expression selectively in nNOS-expressing interneurons, we were able to stimulate MMP activity, potentiate cue-induced reinstatement, and drive reinstatement in the absence of cues. Furthermore, Gq stimulation in nNOS-expressing interneurons induced MMP activity throughout the accumbens, and this activity was completely abolished by an nNOS inhibitor. Future experiments will determine the effects of this activity on synaptic strength, measured by AMPA:NMDA ratio. Together, these data indicate that nNOS-expressing interneurons are a novel portal for cortical regulation of cocaine-seeking, and furthermore that they may constitute a 'master switch' for plasticity on medium spiny neurons that underlies relapse to drug seeking.

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51.06/F42. ***Electrochemical detection of glutamate- and Gq-dreadd-evoked nitric oxide release in the nucleus accumbens***

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The gaseous transmitter nitric oxide (NO) is produced in the nucleus accumbens core (NAcore) by a subpopulation of interneurons that express neuronal nitric oxide synthase (nNOS). Among other things, NO plays a critical role in the nitrosylation and activation of matrix metalloproteinases (MMPs) required for dendritic spine head (dh) expansion on medium spiny neurons (MSNs), linked to cue-induced cocaine seeking. We have shown that cocaine exposure enhances activity of the nNOS enzyme in the NAcore leading to the nitrosylation of MMPs, while inhibition of nNOS inhibits cue-induced activation of MMPs and cocaine seeking. Further, we show here that infusion of the mGluR5 agonist CHPG into the NAcore produced reinstated drug seeking, which was blocked by the co-infusion of nNOS inhibitor N-Propyl-L-Arginine (NPLA). In order to validate that activation of NAcore nitrgergic interneurons translates to NO release in real time, evoked NO levels were measured in anesthetized animals using Nafion + o-PD coated S2 multi electrode arrays and the Quanteon FAST16mkII system. Puff application of glutamate or CHPG produced a reproducible dose-dependent increase in NO release in the NAcore, which was inhibited by the mGluR5 antagonist MTEP or NPLA, respectively. Moreover, NO efflux was dose-dependently evoked by stimulation of Gq-coupled designer receptors exclusively activated by designer drugs (DREADDs), selectively expressed in NAcore nitrgergic interneurons. Taken together, our results demonstrate that activation of glutamate receptors (including mGluR5) in the NAcore produced NO release. Further, we show that activation of Gq-signaling specifically in NAcore nitrgergic interneurons also induced NO release. Combined, these data indicate that activation of nitrgergic interneurons, and the subsequent NO release, is a crucial step in the signal transduction cascade between cue-induced glutamate release in the NAcore and the activation of MMPs and increased dh associated with cued cocaine seeking.

51.07/F43. ***Integrins and integrin linked kinase as a signaling pathway for mmp-9 induction of transient synaptic plasticity in cocaine relapse***

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Chronic cocaine exposure produces neuroplasticity within the nucleus accumbens core (NAcore) that leads to increased vulnerability to relapse, even after protracted abstinence. Matrix metalloproteinases (MMPs) are inducible endopeptidases that degrade extracellular matrix (ECM) proteins (such as fibronectin, laminin and thrombospondin), as well as non-ECM signaling molecules, and reveal an RGD domain that binds and signals through integrins. Integrins are heterodimeric transmembrane cell adhesion receptors composed of subunits  $\alpha\beta$ , and their primary signaling kinase is the integrin linked kinase (ILK). A variety of data support integrin in the accumbens as a possible signaling mechanism for addictive behaviors. Previous results from our lab show that  $\beta3$  integrin is upregulated by cocaine self-administration and its stimulation promotes changes in spine morphology and AMPA receptor trafficking. Moreover, administering an RGD-containing peptide into the NAcore inhibits cocaine-induced reinstatement. It has also been shown by others that MMP-9 activation produces changes in spine morphology and NMDA receptor surface diffusion by signaling through  $\beta1$  integrins in the hippocampus. We know that chronic cocaine exposure increases activity of MMP-9 and this promotes transient synaptic plasticity (t-SP: increases in spine head diameter and AMPA/NMDA) and reinstatement of drug seeking. We hypothesized that  $\beta1$  and  $\beta3$  integrin signaling through ILK are important in order to promote synaptic growth and regulate actin polymerization and AMPA receptor trafficking during t-SP. In order to support this hypothesis, we reduced integrin and ILK protein using an antisense morpholino and evaluated the capacity of both to mediate cue-reinstated behavior. Our preliminary studies with knock-down of NAcore levels of B1 integrin and ILK is contrary to our hypothesis, and a reduction in cue-induced reinstatement of cocaine seeking was observed in morpholino treated versus control rats. However, this may occur because the knockdown of B1 integrin caused a compensatory upregulation of ILK. We are conducting further studies us small molecule antagonists of ILK to evaluate the role of integrin signaling on reinstated cocaine seeking.

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51.08/F44. ***Overexpression of acid-sensing ion channel 1A in the nucleus accumbens core potentiates cocaine-seeking, but not food-seeking, behavior in rats***

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Recent evidence indicates that acid-sensing ion channels (ASICs) in the nucleus accumbens influence conditioned place preference for cocaine and cocaine self-administration. However, whether ASICs are involved in regulating cocaine-seeking behavior is unknown. To address this issue, male Sprague-Dawley

rats underwent surgery for intravenous catheter implantation and implantation of bilateral cannulas aimed at the nucleus accumbens core. The rats then underwent cocaine self-administration for a minimum of 12 d, in which active lever presses produced an intravenous infusion of cocaine and a light-tone combination of cues. Upon completion of self-administration, rats received microinjections of adeno-associated virus (AAV) into the nucleus accumbens core to produce overexpression of the ASIC1A subunit or GFP alone (control group). Groups were matched based on self-administration behavior (cocaine infusions and active lever pressing). The rats then remained in their homecages for 3 weeks in order to ensure robust ASIC1A overexpression as well as mimic procedures necessary for the incubation of craving. Rats were then returned to the operant chambers to begin drug-seeking testing. In the first session, rats underwent a cue-induced drug-seeking session, in which active lever presses produced the cocaine-associated cues. ASIC1A overexpression induced significantly more active lever pressing than their control GFP-alone counterparts. Rats then underwent minimum of d of extinction, during which active lever presses had no consequences, in order to extinguish their lever pressing. Rats overexpressing ASIC1A had more active lever presses on day 1 of extinction and required more extinction sessions in order to reach criterion. Subsequent reinstatement testing using either cues, a cocaine-prime, or a combination of cues with a cocaine-prime revealed similar effects, as rats overexpressing ASIC1A had higher levels of cocaine-seeking behavior. A subsequent experiment replicated the behavioral procedures but with food self-administration and food-seeking behavior. In this case, ASIC1A overexpression in the nucleus accumbens core had no effect on any measure of food-seeking behavior. Together, these findings indicate that overexpression of ASIC1A in the nucleus accumbens core potentiates cocaine-seeking behavior and that this effect does not appear to generalize to non-drug reward-seeking behavior. The present results suggest that targeting ASICs, and particularly the ASIC1A subunit, may be an effective and selective method for altering cocaine seeking.

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**51.09/G1. *Systemic administration of a kainate receptor antagonist attenuates cocaine seeking and alcohol preference in rats***

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Cocaine addiction in the United States continues to be a large public health concern for which there are no FDA-approved pharmacological treatments. Ionotropic glutamate receptors play an important role in cocaine-seeking behavior. To date, the vast majority of studies of the role of ionotropic glutamate receptors in cocaine addiction have been limited to AMPA receptor function primarily because of a lack of available drugs that selectively target kainate receptors. Thus, the role of kainate receptors in cocaine-seeking behavior remains unclear. Here, we examined the role of kainate receptors in cocaine seeking using novel pharmacological antagonists of kainate receptors. Rats were allowed to self-administer cocaine (0.25 mg/infusion i.v.) for 2 days. Cocaine self-administration was extinguished by replacing cocaine with saline. Rats were then given an acute systemic injection of cocaine (10 mg/kg, i.p.) to assess their drug-seeking behavior. In subsequent reinstatement tests, rats were pretreated with

the selective kainate receptor antagonist UBP302 (0.5, 1, 5, 10 mg/kg, i.p.) or LY466195 (4, 10 mg/kg, i.p.) followed by a priming injection of cocaine. Systemic administration of LY466195 attenuated the ability of an acute priming injection of cocaine to reinstate drug-seeking behavior. In a separate set of experiments, the effect of LY466195 on alcohol consumption was assessed using the intermittent two-bottle choice paradigm. Preliminary results indicate that LY466195 (4, 10 mg/kg, i.p.) administration showed a strong trend towards reducing alcohol preference. Together, these results suggest that kainate receptor antagonists may be useful in the treatment of cocaine craving and may influence alcohol intake as well.

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51.10/G2. ***The role of projections from the nucleus accumbens shell to the ventral pallidum in mood and motivation for cocaine***

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Cocaine users often cite negative affect as a key factor behind relapse. However, the relevant neurocircuitry behind mood and motivational changes in cocaine addiction, and their relationship to one another, is not fully understood. We have recently shown that selective stimulation of nucleus accumbens shell (NAcSh) neural projections to the lateral hypothalamus (LH) increases motivation for cocaine, while producing depression-like despair in rats trained to self-administer cocaine. Global stimulation of NAcSh cell bodies also produces despair, but decreases motivation for cocaine, suggesting that other NAcSh outputs may override the motivational effects of NAcSh-LH terminal stimulation. Activity in NAc projections to ventral pallidum (VP) has been associated with depressed mood in drug naïve animals, but with conflicting effects on the motivation for cocaine. In the present study, we used a target-specific optogenetic approach to selectively activate NAcSh projections to the VP in male rats. We tested the hypothesis that increased activity in the NAcSh-VP depresses both mood and motivation for cocaine, and, thus, is behaviorally differentiated from the effects of NAcSh-LH projections. Rats were bilaterally injected with either AAV2-hSyn-hChR2(H134)-EYFP or AAV2-hSyn-EYFP control virus into the NAcSh, and implanted with optic fibers in the terminal fields of the NAcSh in the VP. Rats were trained to self-administer intravenous cocaine (0.5 mg/kg/infusion, i.v.) h/day for weeks. We measured the effect of laser stimulation of the NAcSh-VP pathway (30 min pretreatment, 10 sec/min, 20 Hz, 50 mW) on motivation for cocaine as assessed by 1) performance on a progressive ratio (PR) schedule of reinforcement for cocaine, 2) drug-paired lever presses under extinction conditions and 3) cocaine-primed reinstatement. We measured behavioral despair and anhedonia with the forced swim and sucrose preference tests, respectively. Optogenetic stimulation of NAcSh-VP terminals significantly decreased the effort to self-administer cocaine in ChR2 animals compared to eYFP controls, as indicated by 43.6% lower breakpoints. ChR2 animals also had a 43.6% reduction in lever presses during early extinction and 32.9% decrease during reinstatement compared to eYFP controls. However, in contrast to global stimulation of the NAcSh, we found no differences in measures of immobility in the forced swim test, or a difference in preference for a 1% sucrose solution. These findings suggest that activity in

the NAcSh-VP circuit may decrease motivation for cocaine independent of changes in mood, and thus may serve as a possible neural substrate for addiction treatment.

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51.11/G3. ***Loss of BDNF-TrkB-PLC signaling in accumbens shell neurons attenuates cocaine-induced dendritic spine formation***

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Chronic cocaine induces dendritic spine growth in accumbens shell (NACsh) neurons, but this effect has not been shown to directly enhance addictive behavior. Spine formation has been functionally linked to BDNF-TrkB signaling in other brain regions, but whether this mechanism underlies cocaine-induced spine formation is unknown. We previously found that cocaine induces BDNF activation of TrkB signaling through PLC. In this study, we tested the necessity of BDNF-TrkB-PLC signaling on dendritic spine formation in NACsh neurons, and compared effects with modulation of cocaine self-administration behavior. We constructed a novel HSV bicistronic vector expressing both GFP and a mutated TrkB that selectively blocks endogenous TrkB-PLC signaling (HSV-TrkB<sup>Y816F</sup>), while preserving other TrkB signaling pathways. Rats implanted with bilateral NACsh cannulae were trained to self-administer (SA) cocaine on a fixed ratio (FR) schedule for 3-4 weeks, and dose-response for cocaine SA was assessed before, during, and after transient HSV expression TrkB<sup>Y816F</sup> or GFP-only controls. A second HSV infusion was followed by assessment of motivation for cocaine on a progressive ratio reinforcement schedule (PR). For morphological analysis, separate cohorts engaged in cocaine or saline SA for 3 weeks, and HSVs were infused into the NACsh followed by 2 more days of SA and 24 h withdrawal prior to brain perfusion. Dendritic spine densities were quantified from confocal images of GFP-labeled neurons using Volocity 3D analysis. TrkB<sup>Y816F</sup> expression caused a transient leftward shift in the dose threshold necessary to maintain cocaine SA compared with GFP controls, indicating increased sensitivity to cocaine reinforcement with loss of endogenous TrkB-PLC signaling. TrkB<sup>Y816F</sup> expression also increased motivation for cocaine as assessed by breakpoints on the PR reinforcement schedule. Chronic cocaine SA increased dendritic spine densities in NACsh neurons compared to tissue from saline SA animals, similar to previous reports. In contrast, TrkB<sup>Y816F</sup> expression during SA reversed cocaine-induced increases in spine density without affecting basal spine density in saline SA animals. These results are the first to implicate BDNF-TrkB activity in cocaine-induced morphological changes in the NACsh, and suggest that the TrkB-PLC signaling pathway is important for this effect. Since inhibiting this TrkB-PLC pathway also enhances the motivation for cocaine, cocaine-induced dendritic spine formation may not functionally contribute to cocaine addiction, and could represent a counter-adaptation process that reduces addictive behavior.

51.13/G5. ***Riluzole impairs reinstatement to cocaine seeking***

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Cocaine abuse alters cellular dynamics within several regions of the brain's reward circuitry, including the ventral tegmental area, nucleus accumbens and prefrontal cortex (PFC), among others. Elucidation of these cellular adaptations can help identify pharmacotherapeutic candidates for cocaine addiction. One example of such a candidate is the glutamate transporter GLT-1/EAAT2, which is decreased in the nucleus accumbens following cocaine experience. GLT-1 is responsible for approximately 90% of glutamate uptake in the brain, and is critical for neuroprotection and fidelity of synaptic processing. Previous studies have reported that compounds which restore expression of GLT-1 can also reduce behavioral measures of drug seeking. Thus, we wished to test the hypothesis that another known regulator of GLT-1, riluzole, might also reduce cocaine seeking. Riluzole is an FDA approved drug for Amyotrophic Lateral Sclerosis (ALS), which decreases neuronal activity by blocking voltage-gated sodium channels. In addition, riluzole upregulates expression of GLT-1 in vitro and in vivo, leading to increased glutamate uptake by astrocytes. To determine whether riluzole has an effect on cocaine seeking, we employed the rat self-administration/extinction/reinstatement model of cocaine abuse. During the extinction phase, rats received chronic intraperitoneal injections of vehicle or riluzole (1 or 5 mg/kg), thirty min before each session. We observed a dose-dependent reduction in cue- and cocaine-primed reinstatement to cocaine. However, riluzole had no effect on cue-primed reinstatement of sucrose seeking. In addition, we recorded intrinsic excitability in prefrontal cortical neurons using whole-cell patch clamp electrophysiology in slices of rats trained to self-administer cocaine or saline, receiving chronic riluzole or vehicle injections during extinction. Preliminary data indicate a cocaine-dependent increase in prelimbic neuron excitability, which is reversed by administration of riluzole. Surprisingly, preliminary results also indicate that riluzole administration results in an increase in excitability in infralimbic neurons. These results suggest that riluzole restores levels of intrinsic excitability in the prefrontal cortex, which may contribute to its effect on cocaine seeking. These results further support an existing body of literature which implicates GLT-1 regulators as therapeutic candidates for psychostimulant addiction.

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51.14/G6. ***Regulation of glutamate transporter-1 gene expression by cocaine self-administration and withdrawal***

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Relapse to cocaine abuse is characterized by alterations in glutamatergic signaling in the nucleus accumbens (NAc). For example, glutamate levels are increased in the NAc during reinstatement to

cocaine seeking in the rat. Glutamate uptake is maintained primarily by the astroglial glutamate transporter GLT-1, which accounts for up to 90% of glutamate clearance from the synapse. GLT-1 protein expression and function is decreased in the NAc following cocaine self-administration and withdrawal, and this decrease is correlated with both cocaine exposure and length of withdrawal. Moreover, restored expression of GLT-1 is a necessary component of compounds which block reinstatement to cocaine, including N-acetylcysteine and propentofylline. However, the mechanism responsible for regulation of GLT-1 protein by cocaine remains unclear. To examine this question, rats were trained to self-administer IV cocaine or saline in short access (SA, 15 minutes) or long access (LA, 6 hours) paradigm. Following 2 weeks of SA to cocaine (n=8) or saline (n=8), rats underwent extinction training in the operant chambers for 3 weeks. In contrast, following 10 days of LA to cocaine (n=8) or saline (n=7), rats remained abstinent in the home cage for 45 days. Twenty four hours following the last extinction session (SA rats) or last day of abstinence (LA rats), tissue was harvested from the NAc and prelimbic cortex (PL), a region known to have important projections to the NAc. Gene expression was then examined in these two regions using qRT-PCR and primers specific for two GLT-1 splice variants (GLT-1A and GLT-1B). In SA rats, no differences in GLT-1A or GLT-1B mRNA levels were found between cocaine and saline rats in NAc or PL samples. In the NAc of LA rats, a significant decrease was found in GLT-1A gene expression in cocaine vs. saline rats ( $t(13) = 2.735, p = .05$ ), but no difference was found in GLT-1B gene expression. In the PL of LA rats, we observed a trend toward decrease in GLT-1A and a statistically significant decrease in GLT-1B gene expression ( $t(13) = 2.527, p = .05$ ). These results show that the decrease in GLT-1 protein after SA to cocaine and extinction may not be due to decrease in gene expression, and may reflect protein degradation and/or trafficking. Furthermore, prolonged abstinence after LA cocaine self-administration induces specific changes in GLT-1 gene expression that differs amongst the GLT-1 splice variants, and differs between PL and NAc. Future studies will be designed to investigate the mechanism of the identified genetic suppression of GLT-1 following LA to cocaine.

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**51.16/G8. Deep brain stimulation (DBS) of nucleus accumbens afferent structures attenuates the reinstatement of cocaine seeking**

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Cocaine abuse is a major public health concern, with more than 2 million current users in the United States alone. One of the major obstacles in treating cocaine addiction is the discouragingly high rate of relapse after detoxification. Drug craving and relapse to cocaine seeking are modeled in rodents using the reinstatement paradigm. Deep brain stimulation (DBS) is an FDA-approved treatment for movement disorders. The success of DBS in treating movement disorders has paved the way for its examination as a therapeutic modality in many psychiatric disorders, including drug addiction. Previous work has shown that DBS of the nucleus accumbens shell attenuates priming- and cue-induced reinstatement of cocaine seeking, likely due to modulation of efferent glutamatergic projections from the mesocorticolimbic

system. The medial prefrontal cortex (mPFC), ventral hippocampus (vHipp), and basolateral amygdala (BLA) send strong glutamatergic projections to the nucleus accumbens and have been shown to be critically involved in cocaine seeking. Here, we investigated the effects of DBS in these nuclei on the reinstatement of cocaine seeking. Initially, rats were allowed to press a lever for cocaine (0.254 mg/59  $\mu$ L, i.v.) using fixed-ratio 5 (FR5) schedule of reinforcement. After 21 days of cocaine self-administration, responding was extinguished by substituting saline for cocaine. Following extinction, we assessed cocaine priming-induced reinstatement of drug seeking. During reinstatement test sessions, DBS was administered bilaterally to each nucleus through bipolar stainless steel electrodes. Our findings show that DBS of the mPFC, vHipp, and BLA attenuates the reinstatement of cocaine seeking in rats. These results suggest a role for DBS as a possible therapeutic modality for cocaine addiction and relapse.

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51.17/G9. ***Cav1.2 channel-mediated regulation of GluA1 phosphorylation and trafficking in the hippocampus is essential for extinction of cocaine conditioned place preference***

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Cocaine addiction is a chronic, life-long relapsing disorder. To date, no treatments have been effective in extinction of cocaine seeking behavior. Cav1.2 L-type Ca<sup>2+</sup> channels (LTCCs) are necessary for cocaine-induced behaviors, LTP and long-term memory. Cocaine exposure also modifies glutamatergic synapses in regions innervated by dopaminergic inputs through the regulated trafficking of AMPA receptor (AMPA) localization. Trafficking of the GluA1 AMPAR subunit to the neuronal postsynaptic density (PSD) is regulated by changes in phosphorylation at its serine 831 (S831) residue, a CaM kinase II (CaMKII)-dependent site and its serine 845 (S845) residue, a protein kinase A (PKA) site. Previous findings from our lab has shown that Cav1.2 LTCCs increase cell surface GluA1 levels via CaMKII phosphorylation of S831 in the nucleus accumbens of cocaine sensitized mice. However, the role of Cav1.2 and Cav1.2-mediated GluA1 trafficking in extinction of cocaine seeking behavior remains unknown. Thus, in this study, we employed the cocaine conditioned preference (CPP) model in combination with genetic and molecular techniques to address this question. Using mice lacking Cav1.2 in dopamine D1-containing neurons we found Cav1.2 in the hippocampus to be required for extinction of cocaine CPP. Site-specific knockout of Cav1.2 using AAV-Cre identified a role of Cav1.2 specifically in the hippocampus in cocaine extinction. In cocaine extinguished C57BL/6 mice, there was an increase in S831 P-GluA1 and total GluA1 levels at the PSD in the hippocampus with no change in S845. To directly test the requirement of GluA1 phosphorylation in cocaine extinction, phosphomutant mice with a serine to alanine substitution (S831A or S845A) were utilized. While both genotypes acquired cocaine CPP, both S831A and S845A phosphomutant mice were unable to extinguish cocaine place preference, demonstrating a critical role for S831 and S845 in cocaine extinction. Subcellular fractionation of hippocampus tissue and western blot analyses found no difference in total GluA1 levels in the

cytoplasmic fractions of S831A and S845A mutant mice compared to their respective wild-type littermates. However, lower levels of GluA1 were found in the synaptosomal fractions of both phosphomutant mice compared to wild-type littermates. Experiments examining GluA1 levels in PSD fractions and intracellular mechanisms of Cav1.2 regulation of GluA1 trafficking are currently ongoing. In summary, we have identified a role of Cav1.2 channels in dopamine D1 receptor-containing neurons of the hippocampus in extinction of cocaine CPP via regulation of GluA1 phosphorylation-dependent trafficking.

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**51.18/G10. *Cav1.3 L-type Ca<sup>2+</sup> channels: Role in VTA dopamine neurons on cocaine's behavioral effects and genetic variants in cocaine dependent humans***

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It is well established that Ca<sup>2+</sup> neurotransmission through glutamate receptors within the reward pathway regulates cocaine addiction-related behaviors. Most recently, another route of calcium neurotransmission through L-type calcium channels (LTCCs), has been gaining prominence in the field of addiction. Two subtypes of LTCCs are expressed in the brain, Cav1.2 and Cav1.3, important mediators of CREB activation and CREB-mediated gene expression. We previously reported that Cav1.2 channels mediate the expression of cocaine psychomotor sensitization while Cav1.3 channels, the development (Giordano et al. 2010). In addition, we showed that nifedipine (LTCC blocker) attenuates acute cocaine-induced phosphorylation of CREB (P-CREB) in the nucleus accumbens. However, the specific contribution of the two LTCC subtypes in other cocaine behavioral protocols with greater clinical relevance, like cocaine conditioned place preference (CPP) and cocaine self-administration still remains unknown. Since it has been found that Cav1.3 is more abundant in the VTA (Rajadhyaksha et al. 2004), we investigated the role of VTA Cav1.3 in cocaine CPP. In the present study, using mutant mice expressing 1,4-dihydropyridines (DHP)-insensitive Cav1.2 (Cav1.2DHP<sup>-/-</sup>) treated with nifedipine or Cav1.3shRNA in the VTA, we found that Cav1.3 is necessary for the acquisition of cocaine CPP and not consolidation or expression. Molecular studies have revealed that Cav1.3 mediates acute cocaine-induced P-CREB in the VTA via activation of CaMKinase II. Treatment with KN93 (CaM kinase II inhibitor) in the VTA of wild type C57BL/6J mice blocked the acquisition of cocaine CPP. Additional studies are currently underway to identify the pathway that signals from Cav1.3/CaMKII to CREB in response to cocaine. Recently, it has been shown in neuronal cultures that Ca<sup>2+</sup> influx via Cav1.3 translocates  $\gamma$ CaMKII into the nucleus to shuttle Ca<sup>2+</sup>/CaM for the induction of P-CREB (Huan Ma et al. 2014). Our preliminary results find that acute cocaine increases nuclear levels of  $\gamma$ CaMKII and P-CREB in the VTA. Ongoing studies are examining the role of Cav1.3 channels in cocaine-induced nuclear translocation of  $\gamma$ CaMKII. Our preclinical Cav1.3 findings in cocaine behaviors is further supported by human GWAS study. Examination of 947 single nucleotide polymorphisms (SNPs) within the gene for Cav1.3 (CACNA1D) has identified three significant

SNPs associated with cocaine dependence. Taken together, these findings suggest that VTA Cav1.3 channel-activated pathway plays an important role for the acquisition of cocaine CPP, a measure of cocaine reward that may contribute to cocaine addiction seen in humans.

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51.19/G11. ***L-type calcium channels in the ventral tegmental area mediate cue-induced cocaine-seeking***

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Exposure to drug associated cues promotes drug-seeking behavior and is a challenge for the treatment of drug addiction. The presentation of cues induces burst firing in ventral tegmental area (VTA) DA neurons and subsequent phasic DA release in the nucleus accumbens (NAc) that promotes drug-seeking behavior. Nicotinic and muscarinic receptors in the VTA are critical regulators of burst firing in the VTA and phasic DA release in NAc. Recent data from our laboratory has demonstrated that pharmacological blockade of VTA cholinergic receptors decreases phasic DA release in NAc and reduces cue-induced drug seeking in cocaine withdrawn rats. Importantly, L-type calcium channels (LTCCs) are also expressed on VTA DA neurons and interact with cholinergic receptors to regulate burst firing of VTA DA neurons. However, the role of VTA LTCCs in cue-induced drug seeking are unknown. Here, we sought to determine if pharmacological blockade of LTCCs in the VTA would alter cue-induced drug-seeking following cocaine withdrawal. Male Sprague-Dawley rats underwent 10 days of IV cocaine (0.5 mg/kg/inf) self-administration training, where active lever response resulted in intravenous cocaine delivery in the presence of a compound cue (tone + light), and inactive lever response had no programmed consequence. Following a withdrawal period of 10 days, with no exposure to cocaine or the cues, rats were tested for cue-induced cocaine-seeking, where active lever response resulted in the presentation of the compound cue alone, in absence of cocaine delivery. VTA infusion of the LTCC antagonist, nifedipine (10 µg/side) reduced cue-induced cocaine seeking on withdrawal day 10 (WD 10). In parallel experiments, where male Sprague-Dawley rats were trained to self-administer sucrose pellets, VTA infusion of nifedipine (10 µg/side) on WD10, had no effect on cue-induced sucrose seeking. In subsequent experiments, our data revealed that VTA administration of the LTCC antagonist, isradipine (288 pg/side), also reduced cue-induced cocaine-seeking on WD10. Our results thus far suggest that blockade of L-type calcium channels in VTA specifically reduces cue-induced seeking for the drug reinforcer, cocaine, but not the natural reinforcer, sucrose. Ongoing experiments are seeking to identify the role of specific LTCC subtypes and to determine whether these effects are mediated through regulation of phasic DA release.

**51.20/G12. *Role of intra-accumbens brain-derived neurotrophic factor on cue-induced reinstatement after cocaine self-administration***

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Brain-derived Neurotrophic Factor (BDNF) has been shown to have a critical role not only on neurite growth during early stages of development, but also on physiological functions in the adult brain, as well as on maladaptive behaviors like addiction. Several studies found in the literature explored the role of BDNF in addiction-related brain regions, like the pre-frontal cortex (PFC), the ventral tegmental area (VTA) or the nucleus accumbens, both shell and core (NAcore). In adulthood, the expression of BDNF in the NAcore is low, and the two main sources of BDNF are glutamatergic projections from the PFC and dopaminergic input from the VTA. Both D1- and D2-receptors expressing medium spiny neurons (MSNs) of the NAcore express the primary receptor for BDNF, TrkB. BDNF binding to TrkB induces activation of several intracellular signaling cascades like MAPK, PI3K, phospholipase C- $\gamma$ . It has been proposed that BDNF affects cocaine reward are mainly due to activation of TrkB on D2-expressing MSNs, since specific TrkB gene deletion induces a decrease in cocaine-induced place preference and profound neuronal firing modifications (Lobo et al., 2010). Here we seek to understand the rapid, acute effects of BDNF in the NAcore on drug seeking, using the behavioral model of cocaine self-administration in rats. To study the non-transcriptional effects of BDNF in the NAcore, we microinjected BDNF 15 min before cue-induced reinstatement. BDNF induced a clear decrease of reinstatement that seemed endure for days after administration. Conversely, we used TrkB/Fc, a soluble fusion protein that blocks BDNF binding to TrkB, to test whether blocking endogenous BDNF-induced activation of TrkB could prevent this effect. Preliminary data shows that blocking TrkB activation 15min before reinstatement potentiates reinstatement and prevents co-administration of BDNF from antagonizing reinstated cocaine seeking. These results suggest that, in addition to the long lasting transcriptional effects of BDNF shown in literature, acute activation of the TrkB intracellular pathway just before reinstatement can prevent cocaine seeking.

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**51.21/G13. *Fluoxetine potentiates methylphenidate-induced behavioral stereotypies and subsequent cocaine self-administration in rats***

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The psychostimulant methylphenidate (Ritalin) is used in the treatment of attention-deficit hyperactivity disorder (ADHD) and as a cognitive enhancer in the healthy. Methylphenidate, like cocaine, acts by blocking the reuptake of dopamine. However, unlike cocaine, methylphenidate does not affect serotonin. Serotonin contributes to addiction-related gene regulation and behavior induced by cocaine.

Thus, the lack of serotonin effect may explain methylphenidate's more moderate gene regulation and addiction liability. Indeed, our previous studies showed that enhancing serotonin, by adding selective serotonin reuptake inhibitor (SSRI), fluoxetine, to methylphenidate, potentiates methylphenidate-induced gene regulation in the striatum and nucleus accumbens, mimicking cocaine effects. Here, we investigated behavioral correlates of these neuronal changes in adult rats. Behavior was assessed during repeated drug pretreatment phase (6-8 days) and in response to cocaine, two weeks later. Our results show that adding fluoxetine (5 mg/kg) to methylphenidate (5 mg/kg) potentiates the increase in stereotypies over the course of the repeated pretreatment phase. This effect was particularly pronounced in a subset of rats (about 40%), which showed emerging stereotypies early-on during pretreatment. Two weeks after the pretreatment phase, rats were either given a cocaine challenge (15 mg/kg) and tested in an openfield test or started cocaine self-administration training (150 µg/kg per infusion, 2 h per day for 10 days). In the openfield test, cocaine-induced stereotypies correlated with stereotypies during pretreatment. Moreover, pretreatment with methylphenidate plus fluoxetine facilitated the acquisition of cocaine self-administration. This effect was limited to animals that showed early development of stereotypies during pretreatment. These results show enhanced behavioral responsiveness to cocaine (stereotypies, and cocaine self-administration) in subpopulation of rats after exposure to methylphenidate plus fluoxetine. Our findings suggest that SSRIs may enhance the addiction liability of methylphenidate in a subpopulation of individuals.

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51.22/G14. ***Food restriction stress enhances cocaine seeking and VTA dopamine neuron activity***

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Stress augments drug consumption, sensitizes animals to locomotor activation of psychomotor stimulants, and induces reinstatement of drug seeking. The interaction of stress and drug use is well documented in humans and animals; however, the effects of repeated mild stressors on drug seeking and activity of dopamine (DA) neurons within the ventral tegmental area (VTA) remain under-studied. We carried out a series of experiments to determine if the repeated mild stressor, food restriction (FR), alters basal activity of VTA DA neurons, and to determine if FR during protracted abstinence from cocaine consumption influences drug seeking behavior by a DA dependent mechanism. In the VTA DA recording experiment, rats were either fed ad libitum (control) or were food-restricted to maintain 90% of baseline body weight (FR) for an extended period of time. We then measured the basal firing rates of VTA DA neurons using in vivo single unit recordings under anesthesia to determine if food restriction alters VTA DA activity. In a separate group of animals, male rats were trained to self-administer cocaine (600ug/kg) i.v. for one week, and were then maintained on either control or FR diets for 9 days. Following this schedule, animals underwent within session extinction/reinstatement (cocaine precipitated) procedure during which we measured drug seeking behavior and locomotion. Prior to this procedure, rats were injected i.p. with either a low dose of quinpirole (decrease DA neuron firing rate) or saline (control) to determine if observed effects were dependent on DA neuron activity. We

discovered that FR leads to increased basal VTA DA activity. We also demonstrated that FR during protracted withdrawal increases drug seeking during extinction and reinstatement, and this increase is blocked by reducing DA neuron activity. This data adds to the literature on the interaction between stressful environmental cues and drug seeking behaviors.

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51.23/G15. ***The LPO: Role on dopaminergic transmission, drug taking, and seeking***

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The lateral preoptic area of the hypothalamus (LPO) projects to the ventral tegmental area (VTA). Activity of neurons in the VTA is important for addiction-related behaviors such as cocaine taking and seeking. The goal of this study was to determine how LPO activation modulates neural response in the VTA, and dopamine responsive behaviors such as cocaine taking and seeking. Optogenetic activation of LPO projections to the VTA decreased the firing rate of GABAergic neurons and increased the firing rate of dopaminergic neurons in the VTA. Disinhibition of the LPO neurons via administration of bicuculline into the LPO facilitated cocaine seeking behavior but had no effect on cocaine taking during self-administration. Our results suggest that the LPO sends inhibitory projections to GABAergic neurons of the VTA, thereby increasing dopaminergic activity. Furthermore, disinhibition of the LPO increases cocaine seeking suggesting projections from the LPO to VTA modulates behavioral responsiveness to cocaine. This data supports the role of LPO as a novel structure involved in cocaine-seeking behavior.

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51.24/G16. ***Pharmacological antagonism of the toll-like receptor 4 attenuates cocaine induced reinstatement***

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Cocaine addiction is a chronic relapsing disorder characterized by persistent perturbations to an organism's homeostatic processes resulting in continued relapse vulnerability. Although considerable attention has been paid to the neuroadaptive consequences of chronic cocaine taking, few studies have examined the role of microglia, the brain's resident immune cells, in cocaine relapse. The Toll-Like Receptor 4 (TLR4) is largely expressed on microglia and is a molecular pattern receptor that recognizes xenobiotics as foreign and induces proinflammatory signaling in the central nervous system. Cocaine binds to the TLR4 complex resulting in the release of the proinflammatory cytokines interleukin-1 beta (IL-1 $\beta$ ), interleukin-6 (IL-6), and nuclear factor of kappa B1A (NF $\kappa$ B1A) in the mesocorticolimbic dopamine system. Here, we used a rodent model of cocaine addiction where male Sprague-Dawley rats self-administered cocaine in 1 daily 2-hour sessions. Following self-administration, animals underwent

extinction training and were challenged with cocaine. In one experiment, tissue punches from the ventral tegmental area (VTA) and nucleus accumbens (NAc) were collected and analyzed for expression of proinflammatory cytokine mRNA. Results indicate that cocaine self-administration enhanced the expression of the proinflammatory cytokine, IL-1 $\beta$ . In another experiment, rats were tested in drug-induced reinstatement paradigm where pharmacological antagonism of the TLR4 receptor with lipopolysaccharide from the bacterium *Rhodobacter sphaeroides* (LPS-RS) was administered locally in the NAc and VTA. The results demonstrate that TLR4 antagonism in either the VTA or the NAc significantly reduced cocaine-primed reinstatement of drug seeking. These results are consistent with the hypothesis that cocaine-induced microglia-dependent proinflammatory signaling is involved in cocaine relapse that is characteristic of drug addiction.

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51.25/G17. ***The role of HCRT1 in the VTA on Dopamine signaling; implications for addiction***

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The hypocretin / orexin (HCRT) system has been recognized to modulate motivated behavior via actions on the mesolimbic dopamine (DA) system. This generally neuroexcitatory peptide system sends extensive projections to numerous reward-related regions including the ventral tegmental area (VTA) where both the HCRT receptor 1 (HCRT1) and HCRT receptor 2 subtypes are found. HCRT peptides have been shown to drive VTA DA cell activity, increase DA responses to cocaine in the nucleus accumbens (NAc), and promote cocaine self-administration, while blockade of HCRT1 produces the opposite effects. Although the existing literature points to the HCRT1 as an important regulator of reward and reinforcement processing, the majority of studies have employed acute, pharmacological manipulations of HCRT signaling. Moreover, these studies have traditionally relied on peripheral delivery of the HCRT1 antagonist, SB-334867. Therefore, currently little is known about the long-term, modulatory role of HCRT1 in the brain, or the specificity of actions at this receptor within the VTA. To address these issues, we knocked down HCRT1 in the VTA and used fast scan cyclic voltammetry to measure baseline and cocaine-induced changes to DA signaling in the NAc. Further, we evaluated the effects of VTA-HCRT1 knockdown on the acquisition and maintenance of cocaine self-administration behavior. Preliminary results suggest that long-term knockdown of VTA-HCRT1 disrupts DA neurotransmission in the NAc under baseline and cocaine conditions, and also reduces cocaine self-administration behavior. When considered in the context of the existing literature, our experimental findings provide further support for the involvement of HCRT1 in the VTA in regulating reward and reinforcement processes, and further suggest that HCRT1 may be an effective target for future pharmacotherapies to treat substance abuse, particularly the abuse of cocaine.

**51.26/G18. *Increased expression of 5-HT6 Receptors in the indirect pathway reduces cocaine self-administration***

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“Increased expression of 5-HT6 Receptors in the indirect pathway reduces cocaine self-administration” Drug addiction affects millions of people throughout the world and contributes heavily to healthcare costs and death. The nucleus accumbens (NAc) in the mammalian ventral striatum plays a large role in addiction, particularly in motivation for drug and reward-seeking behavior. Serotonin (5-HT) neurotransmission has also been implicated in addiction, and 5-HT6 receptors are strongly expressed in the direct and indirect pathway medium spiny neurons (MSNs), the main outputs from the NAc. While there is evidence linking these receptors to drug reward, the exact mechanism by which they influence drug-associated behavior is unknown. In the present study we used viral vectors using dynorphin- or enkephalin promoter to drive expression of 5-HT6 receptors or enhanced green fluorescent protein (eGFP) selectively in the direct or indirect pathway MSNs of the NAc shell (NAcSh), respectively. Rats were then trained to self-administer cocaine and their responding was investigated using fixed ratio, progressive ratio, and dose-response operant reinforcement conditions. Increased 5-HT6 receptor expression in indirect but not direct pathway MSNs changed the overall pattern of cocaine taking, reduced the amount of cocaine self-administered under fixed ratio schedules, especially at low doses, increased the time to the first response and length of the inter-infusion interval, but did not alter motivation as measured by progressive ratio “break point” analysis. We conclude that 5-HT6 receptors in indirect pathway neurons of NAcSh increased the sensitivity to the reinforcing properties of cocaine, particularly at low doses, suggesting that these receptors may be a target for pharmacological manipulation in the treatment of cocaine addiction.

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**51.27/G19. *Phasic dopamine release in the nucleus accumbens during cocaine self-administration under different operant requirements***

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The mesocorticolimbic dopamine system plays a crucial role in the development of drug addiction. Dopamine levels are elevated in response to drug-paired cues in rats self-administering cocaine, though this has been primarily examined only under low effort conditions with a fixed ratio 1 (FR1) reinforcement schedule. A hallmark of drug addiction is the willingness to exert considerable time and effort to obtain the drugs. However, it is not known whether cue-evoked dopamine release is related to the effort exerted in rats self-administering cocaine under high effort reinforcement schedules. We utilized fast-scan cyclic voltammetry to monitor phasic dopamine release using chronically-implanted

electrodes in the nucleus accumbens in rats self-administering cocaine under FR3 and progressive ratio (PR) reinforcement schedules. Upon completion of the required number of nose pokes, rats received an intravenous 300 µg/kg cocaine infusion. Cocaine administration was paired with a 5 second duration audio cue (tone) and 2 second time out period. Our preliminary results illustrate higher dopamine release to cocaine-paired cues during the PR reinforcement schedule sessions relative to the FR3 reinforcement schedule sessions. We also employed a model to estimate the cocaine concentration in the brain during these behavioral sessions to associate the dopamine response to cocaine levels. Preliminary analysis suggests the dopamine-release towards cocaine-paired cues is not related to the cocaine concentration in the brain. These findings suggest that dopamine release toward cocaine-paired cues is sensitive to the amount of effort required to earn a drug infusion, and is not driven by the pharmacological actions of cocaine.

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**52.02/G21. *Blocking infralimbic basic fibroblast growth factor (bFGF or FGF2) facilitates extinction of drug seeking***

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Stimulant drug use results in structural and functional changes in reward-related brain regions (Flores & Stewart, 2000). These changes may underlie the persistence of compulsive drug seeking and relapse that characterizes drug addiction. Neurotrophic factors, such as basic fibroblast growth factor (bFGF or FGF2), are necessary for neuronal survival, growth, and differentiation, and may mediate drug-induced morphological changes that underlie the perseveration of addiction. Following cocaine exposure, bFGF is increased in reward-related brain regions (Fumagalli et al., 2006), including the infralimbic medial prefrontal cortex (IL-mPFC). The IL-mPFC is necessary for extinction (Otis et al., 2014; LaLumiere et al., 2010), but whether drug-induced over-expression of bFGF in this region affects extinction is unknown. Thus, we aimed to determine if blocking bFGF in IL-mPFC would facilitate extinction following cocaine self-administration. Rats were trained to lever press for i.v. infusions of cocaine (0.25mg/inf, 90 min/day) prior to extinction. Extinction consisted of four 30 min extinction sessions, in which rats were infused into the IL-mPFC with a neutralizing antibody against bFGF that blocks the biological function of bFGF, prior to each session. Extinction retention was tested during a subsequent 90 min extinction session. Blocking bFGF in the IL-mPFC decreased lever pressing during the 90 min extinction session, indicating facilitated extinction. In contrast, blocking bFGF alone was not sufficient to facilitate extinction, as blocking bFGF and returning rats to their home cage had no effect on subsequent extinction of drug seeking. Next, we examined if bFGF or its high affinity receptor fibroblast growth factor receptor 1 (FGFR1) protein expression was altered following extinction. Rats were trained to self-administer cocaine as before with half undergoing extinction or not. Additionally, rats that were reinforced with sucrose and underwent extinction or not, rats that received yoked-saline infusions that were paired with cocaine self-administering rats (extinction or not), and naïve home cage control were included. bFGF protein expression in the IL-mPFC was only increased following cocaine self-

administration, an effect reversed by extinction. FGFR1 protein expression was not significantly altered in any group. These results suggest that cocaine-induced over-expression of bFGF in the IL-mPFC inhibits extinction, as reducing bFGF expression during extinction permits rapid extinction. Therefore, targeted reductions in bFGF during therapeutic interventions could enhance treatment outcomes for addiction.

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**52.03/G22. PKA mediates prelimbic neuronal excitability underlying cocaine-associated memory retrieval**

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Persistent drug seeking is maintained by drug-associated memories, through which cues can elicit craving and relapse. Thus, impairing retrieval of drug-associated memories could dampen the motivation to seek drugs. Recently, retrieval of drug-associated memories was shown to be disrupted by inhibition of  $\beta$ -adrenergic receptors ( $\beta$ -ARs) in the prelimbic medial prefrontal cortex (PL-mPFC; Otis et al., 2013).  $\beta$ -ARs stimulate cAMP dependent protein kinase (PKA; Mueller et al., 2008) which enhances neuronal excitability by inhibiting the slow afterhyperpolarization (sAHP; Zhang et al., 2013). However, whether this intracellular signaling cascade underlies retrieval is unknown. Using patch-clamp electrophysiology and the conditioned place preference (CPP) paradigm, we determined whether intrinsic excitability of PL-mPFC neurons maintains memory during retrieval. Rats were conditioned to associate one chamber, but not another, with cocaine. During post-conditioning CPP retention trials, rats had access to both chambers and spent more time in the previously cocaine-paired chamber than in the saline-paired chamber. Microinfusions of the PKA antagonist (Rp-2'-O-MB-cAMPs) in the PL-mPFC before the first retrieval test disrupted expression of the CPP on that trial and all subsequent trials. This retrieval deficit was rescued by co-administration of sAHP antagonist (UCL-2077). In vitro patch-clamp recordings were used to examine the physiological consequences of noradrenergic signaling in the PL-mPFC. Pyramidal cells in the PL-mPFC were dialyzed with Rp-cAMPs or not prior to bath application of norepinephrine (NE). In the absence of Rp-cAMPs, NE increased the number of evoked action potentials by reversing the sAHP to a slow afterdepolarization (sADP). However, Rp-cAMPs blocked the NE-induced reversal of the sAHP, and prevented the increase in evoked action potentials. Finally, we examined whether intrinsic excitability maintains cocaine-associated memory retrieval. Cocaine-conditioned rats were split into low retrieval (LR) and high retrieval (HR) groups based on CPP scores. Patch-clamp recordings from PL-mPFC pyramidal neurons revealed that the number of evoked action potentials was correlated with CPP scores, and was increased in neurons from HR rats compared to LR rats. These data demonstrate that intrinsic neuronal excitability in the PL-mPFC maintains drug-associated memory retrieval through a PKA-dependent signaling cascade. Thus, modulation of the intrinsic excitability of PL-mPFC neurons could have therapeutic potential in the treatment of drug addiction.

**52.04/G23. *The role of medial prefrontal cortex gap junction communication in retrieval and extinction of cocaine seeking***

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Drug-associated cues can trigger craving and relapse for drug addicts. Preventing retrieval of cue-associated memories or reducing cue reactivity through extinction could reduce relapse rates. Retrieval of a cocaine-associated memory is dependent on activation of the prelimbic medial prefrontal cortex (PL-mPFC; Otis et al., 2013) whereas extinction of cocaine seeking is consolidated in the infralimbic medial prefrontal cortex (IL-mPFC; Otis et al., 2014). Although the neurochemical and synaptic mechanisms underlying drug-related behavior have been investigated extensively, little is known regarding the contribution of gap junction communication between neurons and astrocytes. Both neurons and astrocytes express gap junctions. Gap junctions are specialized membrane structures built of connexin channels that allow cytoplasmic continuity between connected cells. Previous work has demonstrated that activity of either neuronal or astrocytic gap junctions can alter neuronal activity and plasticity (Palacios-Prado et al., 2014; Pannasch et al., 2011). Thus, we investigated the role of neuronal gap junctions, astrocytic gap junctions, or the combination of both during retrieval and extinction of cocaine seeking using the conditioned place preference (CPP) model. Following conditioning and prior to the first retrieval (15 min) or extinction (30 min) test, rats received a bilateral microinfusion of either a non-selective gap junction blocker (carbenoxolone), a neuron-specific gap junction blocker (quinine), an astrocyte-specific gap junction blocker (IRL-1620), or vehicle into the PL-mPFC or IL-mPFC and were tested daily. General and astrocytic gap junction blockade in the PL-mPFC initially disrupted retrieval of a CPP, but failed to maintain prolonged disruption as demonstrated by a return of CPP in later test trials compared to extinguished controls. Neuronal gap junction blockade in the PL-mPFC enhanced maintenance of the cocaine-associated memory relative to other groups. General, neuronal, or astrocytic gap junction blockade in the IL-mPFC resulted in disrupted extinction learning. These results suggest that individually or collectively disrupting neuronal and astrocytic gap junction networks in the PL-mPFC or IL-mPFC prolong drug-seeking behavior, further suggesting that gap junction communication in the mPFC may play a critical role in drug-seeking behavior.

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**52.05/G24. *Identification of distinct neuronal ensembles selectively activated by discrete cues associated with cocaine or heroin seeking in rats***

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Background: Learned associations between discrete cues (or contexts) and drug effects are thought to be encoded by sparsely distributed patterns of neurons called “neuronal ensembles”. Here, we

determined whether cocaine- and heroin-related cues are encoded by distinct neuronal ensembles within the medial prefrontal cortex (mPFC) in a rat model of drug relapse. Methods: We trained rats to self-administer cocaine (0.75 mg/kg) and heroin (0.0375 mg/kg) on alternate days (3-h/day; 18-days). Cocaine and heroin infusions were paired with two distinct levers and the presentations of discrete cue-lights (20-sec). After 5 days of forced abstinence, rats were assigned to four groups based on the order of two 5 min extinction tests (spaced by 20-min) with the drug-related cues: Cocaine-Cocaine, Heroin-Heroin, Cocaine-Heroin and Heroin-Cocaine. Non-test control group was trained but kept in the operant chamber on test day. We detected Homer 1a, Arc and Fos mRNA using RNAscope assay. We selected target-probes to detect nuclear signal for Homer 1a- and Arc-activated neurons from the ensembles activated during the first test and second test, respectively. Results: Preliminary analysis showed higher overlap (co-expressing Homer 1 and Arc) in the Cocaine-Cocaine group than in the Heroin-Heroin, Cocaine-Heroin, and Heroin-Heroin groups for both dorsal and ventral mPFC. Furthermore, 92-98% of these Homer 1a- and Arc-positive cells co-expressed Fos mRNA, a common marker of neural activity. Conclusions: Our results suggest that distinct mPFC neuronal ensembles encode two different drug-cue memories. Further experiments are needed to characterize these ensembles and assess mutually exclusive causal roles in drug-seeking behavior.

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52.06/G25. ***Interleukin-1 in the dorsal hippocampus is a novel mediator of acquisition of heroin-conditioned immunosuppression***

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Like many other opiates, heroin suppresses the immune system. Similarly, exposure to environments associated with heroin use can produce immunosuppression through Pavlovian conditioning. The dorsal hippocampus (DH) is necessary for this conditioned effect. Interleukin-1 $\beta$  (IL-1 $\beta$ ), a pro-inflammatory cytokine expressed in the DH, has been linked to learning and memory. Our laboratory has demonstrated that siRNA-mediated IL-1 $\beta$  gene silencing in the DH prevents the expression of heroin-conditioned immunosuppression. Here we investigated the role of DH IL-1 in the acquisition of heroin conditioned immunosuppression. To this end, rats were conditioned to associate heroin (1 mg/kg, SC) with a distinct context. During Pavlovian conditioning, rats received bilateral microinfusions of the endogenous IL-1 antagonist (IL-1RA; 1.25  $\mu$ g/0.5  $\mu$ L/side) or saline into the DH either 3 min before or 2 h after each of context-heroin pairings. To control for the timing of the injections, each group received saline when the other group received IL-1RA. Six days later, rats were either re-exposed to the heroin-paired context for 60 min or remained in their home cages. Immediately after this manipulation, their immune system was challenged using lipopolysaccharide (LPS, 1  $\mu$ g/kg, SC), a component of gram negative bacteria. The rats were sacrificed 6 h later. Splenic iNOS, IL-6, IL-1 $\beta$ , and plasma nitrate/nitrite levels were assessed. IL-1RA microinjection 3 min before, but not 24 h after, context-heroin pairing blocked the acquisition of heroin-conditioned immunosuppression, as indicated by no significant

suppression of splenic proinflammatory responses to LPS after heroin context re-exposure. Future studies will evaluate whether this time-dependent, IL-1RA-induced acquisition deficit is due to a disruption of associative learning or of the primary immune suppressing effects of heroin during training. Regardless of the mechanism, these findings suggest that elements of the IL-1 signaling pathway may be therapeutic targets for restoring immune function in opiate-exposed populations.

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52.08/G27. ***Remembering to abstain: The impact of working memory on length of first quit attempt in drug users***

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Background: Drug addiction continues to be a major public health problem: more than 20 million Americans aged 12 or older use illicit drugs (NCADD). While much attention is given to treatment and prevention, limited attention is given to the work drug users themselves conduct in order to quit. Most drug users have tried to quit using drugs at some point, and most often find themselves unsuccessful. One factor associated with this difficulty may be related to working memory. Recent research has examined working memory in drug users and found poorer working memory than in non-users. To explore this further, the present study aimed to examine the link between working memory and the length of time an individual was able to abstain from substance use during their first quit attempt. Methods: A secondary data analysis of participants enrolled in the NIDA-funded NEURO II-HIV Prevention RCT study in Baltimore, Maryland. The sample included 16 drug-using adults aged 18 to 59 (M age=42.2, SD=9.8). Participants were community-recruited. They completed an HIV-risk behavior interview that included questions about substance use and whether they had previously attempted to quit using drugs. Participants completed a battery of neuropsychological tests which included the WAIS-II digit span test, which measures forward and backward digit span memory. Linear regression was conducted using the raw scores in order to determine the association between digit span and the length of the time of first quit attempt. Results: Linear regression was conducted to compare digit span total score (M=13.78, SD=4.36) and the length of first quit attempt in years (M=2.28, SD=3.24). Digit span total score was significantly associated with length of first quit attempt,  $b = -.22$ ,  $t(165) = -2.88$ ,  $p = .005$ , such that those with stronger working memory ability maintained a longer period of nonuse. The results for the Shipley Institute of Living total score (M=33.7, SD=97.20) were included in these analyses to control for general intellectual functioning. Conclusions: Working memory appears to play a role in how long a person is able to maintain abstinence from drugs and alcohol. Individuals who were able to remember more numbers during the digit span test seemed to be able to abstain longer from drugs and alcohol during their first quit attempt than those who remembered fewer. These findings suggest way in which we can enhance treatment techniques aimed at drug addicts by including memory exercises to improve working memory. Perhaps individuals who are struggling to maintain abstinence may show improved lengths of sobriety--ideally long-term--as working memory is improved.

52.09/G28. ***PP1/GSK3 signaling pathway is involved in the reconsolidation of cocaine reward memory***

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Our previous study demonstrated that glycogen synthase kinase-3 (GSK3) activity is highly induced in nucleus accumbens (NAc), hippocampus, and prefrontal cortex during memory retrieval, and that reconsolidation of cocaine reward memory can be attenuated by inhibition of GSK3. Since protein phosphatase 1 (PP1) is an activator of GSK3 $\beta$  and GSK3 $\beta$  is part of a multi-protein NMDA receptor complex, the roles of PP1 and NMDA receptors in the reconsolidation of cocaine contextual reward memory were investigated in this study. Adult male CD-1 mice underwent cocaine place conditioning for 7 days and were tested for place preference on day 9. Twenty-four hours after the test for place preference, mice were confined to the compartment previously paired with cocaine in a drug-free state for 10 minutes to reactivate cocaine-associated memories. Western blotting indicated that levels of phosphorylated GSK3 $\alpha$ Ser21 and GSK3 $\beta$ Ser9 were down-regulated in the mouse nucleus accumbens and hippocampus after the reactivation of cocaine cue memories, consistent with our previous findings. Interestingly, PP1 inhibition with okadaic acid (OA, 150 ng/3 $\mu$ l, i.c.v) 30 minutes before re-exposure to the compartment previously paired with cocaine prevented the decrease in phosphorylated GSK3 $\alpha/\beta$  in both nucleus accumbens and hippocampus. Furthermore, administration of OA 30 minutes prior to the reactivation of cocaine cue memories abrogated a previously established place preference when tested 2 hours later. Similarly, administration of the NMDA receptor antagonist MK-801 (0.3 mg/kg, i.p.) immediately after re-exposure to cocaine-paired compartment disrupted the previously established place preference, suggesting interference with reconsolidation of cocaine-associated reward memories. The role of nucleus accumbens in the reconsolidation of cocaine associated memories was also investigated in this study. Immediately after the reactivation session, microinjection of SB216763, a selective inhibitor of GSK-3, into nucleus accumbens blocked the reconsolidation of cocaine-associated reward memories. These findings suggest that the dephosphorylation of GSK3 that occurred upon activation of cocaine-associated reward memory may be initiated by the activation of PP1 during the induction of NMDA receptor-dependent reconsolidation of cocaine-related memory. Moreover, the role of PP1 and NMDA receptor in cocaine memory reconsolidation makes them potential therapeutic targets in treatment of cocaine addiction and prevention of relapse.

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52.13/G32. ***Nicotine attenuates the effects of HIV-1 proteins on the neural circuitry of working and contextual memory***

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Human immunodeficiency virus (HIV)-1-associated neurocognitive disorders (HAND) are characterized by synaptic damage and neuronal loss in the brain. Excessive glutamatergic transmission and loss of cholinergic neurons are the major indicators of HAND. By acting as a cholinergic channel modulator, the cognitive-enhancing effect of nicotine in neurodegenerative and cognitive disorders has been documented. However, it remains to be determined whether nicotine has any beneficial effects on memory and synaptic plasticity formation in HAND. In this study, we investigated the effects of nicotine on synaptic plasticity and hippocampus/prefrontal cortex (PFC)/amygdala-dependent memory formation in HIV-1Tg and F34 control rats. Chronic nicotine treatment (0.4 mg/kg/day, s.c.) significantly attenuated the cognitive deficits in both spatial and contextual fear memory in the HIV-1Tg rats, but impaired the contextual learning memory in the F344 rats. To further determine the role of nicotine in the synaptic dysfunction caused by HIV-1 proteins, we analyzed the expression of key representative genes related to synaptic plasticity in the hippocampus, PFC, and amygdala of HIV-1Tg and F34 rats using a custom-designed qRT-PCR array. The HIV-1 proteins significantly altered the glutamate receptor-mediated intracellular calcium cascade and its downstream signaling cascade in a brain region-specific manner. Further, chronic nicotine treatment reversed the effects of the HIV-1 proteins on the expression of genes involved in synaptic plasticity in the three brain regions. The effects of nicotine differed significantly in the HIV-1Tg and F34 rats. Our findings indicate that nicotine can attenuate the effects of viral proteins on cognitive function and produce brain region- and strain-specific effects on the intracellular signaling cascades involved in synaptic plasticity and memory formation. (Supported by DA012844 and DA026356).

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**52.15/G34. *Acute methamphetamine produces long-term deficits in hippocampal-dependent spatial learning and memory retention, decreases PKMzeta, GluA2 and dopamine 1 receptors (D1), and increases microglial expression***

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The purpose of our study was to evaluate the effects of two bolus doses of methamphetamine (MA; 30 mg/kg) on spatial learning and memory. MA is known to have significant effects on dopaminergic terminals within the striatum and also known to decrease Dopamine 1 receptor (D1) expression in the hippocampus (Braren, 2014 *Front. Behav. Neuro.* 4, 438). We evaluated spatial learning and memory using the hippocampal dependent task, the radial 8-arm maze (RAM). The hippocampi from this study were evaluated for the expression of several synaptic markers important for memory and synaptic plasticity. These include the D1 receptor, the atypical protein kinase M zeta (PKM $\zeta$ ), and the AMPA receptor subunit GluA2. PKM $\zeta$  is important for trafficking the GluA2 subunit to the membrane and maintaining GluA2 on the membrane improves memory retention (Migues, 2010 *Nat. Neurosci.* 13, 630-4; Sebastian, 2011 *PLoS One.* 8, e81121). We hypothesize that neuroinflammation plays a role in exacerbating the negative effects of MA-induced memory deficits. One inflammatory marker in particular, COX2, is one of two isoenzymes, which catalyze the conversion of arachidonic acid into

prostaglandins. Several prostaglandins are catalyzed by COX2, but one in particular is very toxic, the prostaglandin J2 (PGJ2). We have found that PGJ2 can induce memory deficits when injected into the hippocampus. Thus, we are interested in determining how MA toxicity may activate this inflammatory pathway involving PGJ2, perpetuating the toxicity leading to sustained cognitive deficits. Our results show that two bolus doses of MA delivered 1 week apart produces deficits in learning the RAM 7 weeks later and decreases PKM $\zeta$ , GluA2 and D1 expression. These results identify the long lasting effects of MA on hippocampal function. Additionally, delivering these two bolus doses of MA after acquiring the RAM memory results in retrieval deficits at 2 weeks after the end of training and results in increased in microglia expression in the CA1 region of the hippocampus. These results elucidate the long lasting learning and memory impairments from acute MA treatment. We hypothesize that these long-term effects of MA are mediated through an inflammatory pathway, as microglia expression is upregulated after MA. Future studies will examine the role of COX2 expression in these samples.

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52.18/G37. ***Chronic fluoxetine ameliorates long-term trace conditioning deficits in mice exposed to chronic nicotine during adolescence***

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Adolescence represents a critical period in which brain areas important for cognition, including the hippocampus and medial prefrontal cortex (mPFC), rapidly develop. As a result, adolescence marks a time when the CNS is sensitive to exogenous insults. Exposure to chronic nicotine during adolescence causes long-term changes in synaptic function, which disrupts cognition. In addition, adolescent nicotine exposure results in cognitive deficits later in life, i.e., in adulthood. In 2014, the CDC reported that 24% of high school students used tobacco products. Given that cognitive deficits are associated with smoking relapse and addiction, long-term cognitive deficits that result from adolescent nicotine exposure may lead to increased risk of nicotine dependence in adulthood. Indeed, adolescent exposure to tobacco increases risk for adult smoking by a factor of 16. Thus, it is important to investigate treatments that ameliorate the long-term cognitive impacts of adolescent nicotine exposure. Here we investigated whether or not chronic fluoxetine (FLX) could ameliorate adolescent chronic nicotine-associated disruption of trace fear conditioning. Trace fear conditioning is a hippocampus-dependent form of associative learning well suited to investigate adolescent nicotine-associated cognitive deficits as it also recruits the mPFC via working memory processes. Chronic FLX was selected as a treatment due to its ability to increase BDNF within the mPFC and hippocampus, as well work showing it can reduce hippocampus-dependent cognitive impairments. Adolescent (PND 38) C57BL/6J mice were administered chronic nicotine or saline via osmotic minipumps for 12 days. Upon pump removal mice were immediately put on FLX treatment via their drinking water (160g/L). After 30 days of FLX treatment, these same mice, now adult aged, were trained in trace fear conditioning and tested 24 hr later with FLX treatment maintained throughout. Mice that received chronic nicotine treatment during adolescence demonstrate deficits in trace fear conditioning during adulthood, while mice that received saline did not.

In addition, chronic FLX ameliorated the nicotine-associated deficit in trace fear conditioning. Our data support previous findings that nicotine administration during adolescence leads to deficits in hippocampus-dependent learning. Furthermore, our data suggest that chronic fluoxetine treatment may be protective against development of long-term cognitive deficits associated with adolescent nicotine exposure. In sum, FLX may be an effective therapy for long-term cognitive deficits associated with adolescent nicotine exposure.

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140.04/G38. ***The role of the rostromedial tegmental nucleus in ethanol-induced conditioned taste aversion in male and female rats***

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The past several decades have witnessed closing of the gender gap in the prevalence of alcohol use disorders (AUDs). In addition, recent work suggests the presence of a telescoping phenomenon, with women developing dependence over a shorter drinking duration and fewer total drinks compared to men. Current theories suggest that a competitive balance between a drug's rewarding and aversive properties changes as drug use progresses from recreational use to addiction. While the involvement of alcohol's rewarding properties in the path to dependence continues to be extensively investigated, the role of alcohol's aversive characteristics has been less well studied, particularly as it relates to differences between the sexes. The rostromedial tegmental nucleus (RMTg) exerts inhibitory control over midbrain dopamine neurons and is involved in encoding aversive stimuli and the behavioral responses to those stimuli. Recent work has also implicated activity within the RMTg in mediating the aversive effects of stimulants. To begin to investigate the role of the RMTg in signaling the aversive properties of alcohol, we measured cFos induction in this brain region following conditioned taste aversion (CTA). Adult male and female Long-Evans rats were exposed to three pairings of novel 0.1% saccharin solution followed by an i.p. injection of 20 ml/kg 0.15 M lithium chloride (LiCl), 1.5 g/kg 20% ethanol (EtOH) or saline. Ninety minutes after fourth exposure to saccharin only, the rats were sacrificed and brains processed for cFos immunohistochemistry. Both LiCl and EtOH produced significant CTA of equal magnitude between males and females compared to saline ( $p < 0.01$ ). LiCl-induced CTA was significantly stronger than EtOH-induced CTA in both sexes ( $p < 0.01$ ). Both LiCl- and EtOH-induced CTA significantly enhanced cFos expression in the RMTg compared to saline ( $p < 0.05$ ). cFos expression was similarly significantly enhanced in the lateral habenula (LHb;  $p < 0.05$ ) - source of prominent glutamatergic input to the RMTg. No significant sex differences in cFos expression were observed. Of note, cFos expression in both the RMTg and LHb were significantly positively correlated with CTA magnitude ( $p < 0.05$ ). In addition, RMTg cFos expression was significantly positively correlated with LHb cFos expression ( $p < 0.01$ ). Together, these data suggest that activity within the RMTg, possibly driven by activity within the LHb, plays role in the aversive properties of drugs, including alcohol, in both male and female rats.

140.07/G41. ***Cellular and synaptic mechanisms of nicotine aversion***

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Nicotine addiction remains a major health problem in the US and throughout the world. Nicotine has rewarding effects at relatively low doses and intensely aversive effects at higher doses. The projection from the medial habenula (MHb) to the interpeduncular nucleus (IPN) contributes to these aversive effects. This “aversive” pathway may influence the development and maintenance of nicotine dependence and also contributes to withdrawal effects. Thus, improved understanding of the mechanisms that underlie the aversive effects of nicotine may be important in developing more effective therapies for smoking cessation. Excitation of the MHb-IPN projections enhances aversion to nicotine, while inhibition of this pathway increases appetitive responding for high doses of nicotine that were previously aversive. Although these results implicate the MHb-IPN circuitry, the downstream post-synaptic targets of the IPN that mediate these effects remain largely uncharacterized. Our hypothesis is that the aversive effects of nicotine occur through an indirect suppression of the excitability of VTA dopamine (DA) neurons. Burst activity in DA neurons is important for reward-associated behaviors, and aversive experiences can suppress DA neuron activity. While the IPN projects to several brain areas, it strongly innervates the lateral dorsal tegmental nucleus (LDTg), a brainstem cholinergic center that controls burst firing of VTA DA neurons. To test the nature of synaptic transmission between IPN projections and LDTg, we expressed Channelrhodopsin (ChR2) in IPN neurons and stimulated the terminals with light while recording specifically from LDTg neurons that project to the VTA. We found that light-evoked synaptic inputs were blocked by the GABA<sub>A</sub> receptor antagonist bicuculline. Additionally, optogenetic stimulation of either the IPN directly, or the IPN terminals in the LDTg specifically, results in aversion. We are testing the modulation of these inhibitory inputs by high and low concentrations of nicotine, and preliminary evidence suggests that high concentrations of nicotine selectively enhance light-evoked GABAergic currents from the IPN onto LDTg neurons that project to the VTA. We also have evidence that optogenetic inhibition of IPN terminals in the LDTg not only reduces aversion to high dose of nicotine, but actually shifts the aversion to reward. These findings highlight the importance of the IPN-LDTg connection in mediating the aversive effects of nicotine and reveal interactions between reward and aversive circuitries that influence overall affective state.

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140.08/G42. ***Septohabenular regulation of nicotine consumption***

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The medial habenula (MHb) projects almost exclusively to the interpeduncular nucleus (IPN) via the fasciculus retroflexus. This major descending projection serves to connect the limbic forebrain and midbrain monoaminergic centers. Recently, our laboratory has shown that the MHb-IPN system, which densely expresses nicotinic acetylcholine receptors (nAChRs), plays an important role in regulating aversive properties of nicotine that limit consumption of the drug. Genetic evidence suggests that deficits in the sensitivity of this system to nicotine increases vulnerability to tobacco dependence. The MHb receives prominent excitatory input almost exclusively from the triangular nucleus of the septum (TNS) and inhibitory input from medial septum (MS) and diagonal band nucleus (NDB). The role of septohabenular pathways in regulating nicotine intake had not yet been explored. Here, we report that TNS neurons are highly sensitive to nicotine in dose-dependent manner, as measured using in vivo single-unit recordings in freely behaving rats. DREADD-mediated excitation of TNS neurons decreased intravenous nicotine self-administration in rats. Conversely, pharmacological blockade of nicotinic signaling in TNS increased nicotine intake. We hypothesized that nicotine stimulates TNS projections to MHb, and that the TNS-MHb pathway inhibits nicotine intake. Surprisingly, DREADD-controlled activation or inhibition of TNS projections to the MHb did not alter nicotine intake. Similarly, modulation of direct TNS projections to the IPN that bypass the MHb did not alter nicotine intake. These data suggest the TNS functions to regulate nicotine consumption independent of its projections to the MHb or IPN. These data reveal the TNS region of the posterior septum as an important neuroanatomical substrate that regulates nicotine intake, but the circuit-level mechanisms through which TNS acts are unknown.

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140.09/G43. ***Effect of DREADD-mediated transient activation of Gq-coupled signaling in lateral habenula neurons on cocaine and food self-administration in rats***

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The lateral habenula (LHb), an epithalamic nucleus located in the dorsal diencephalon is an important regulator of midbrain dopaminergic systems that are known to be involved in the reinforcing properties of cocaine. We previously examined the effect of DREADD (hM4Di) -induced transient activation of Gi/o-coupled signaling in LHb neurons and found that it significantly increased operant cocaine self-administration. Here, we firstly examined the effect of DREADD (hM3Dq)-induced transient activation of Gq coupled signaling on cocaine reinforced operant responding. Male Long-Evans rats were injected with hM3Dq into the LHb and implanted with jugular venous catheters. Approximately 10-12 days after viral infusions, rats were trained to self-administer cocaine (0.75 mg/kg/infusion) on fixed ratio (FR1) reinforcement schedule. Initial results indicate that activation of hM3Dq by the pharmacologically inert synthetic ligand clozapine-N-oxide CNO (1 and 3 mg/kg, i.p) decreases cocaine reinforced operant responding. Secondly, a distinct cohort of rats was infused with viral vectors as described above and trained to self-administer cocaine (0.75 mg/kg/infusion) on progressive ratio reinforcement schedule. CNO-induced activation of hM3Dq significantly decreased operant responding on a progressive ratio

reinforcement schedule. Thirdly, rats infused with hM3Dq into the LHb were trained to self-administer 4 mg food pellets on either FR or progressive ratio reinforcement schedule. Interestingly, our initial results indicate that this manipulation decreases food reinforced operant responding on both FR1 and progressive ratio reinforcement schedules. To determine if the observed effects are due to locomotor depression, we measured locomotor activity in rats previously infused with hM3Dq into the LHb. Following CNO (1 and 3 mg/kg) injections we found a decrease in locomotor activity at baseline levels as well as upon challenge with an acute injection of cocaine (10 mg/kg). Taken together, our results suggest that DREADD-mediated transient activation of Gq-coupled signaling in the LHb decreases operant cocaine and food self-administration and that these effects may, in part, be due to deficits in locomotor activity.

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140.10/G44. ***Cocaine and cue encoding in the rodent entopeduncular nucleus***

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Previous studies have shown that lateral habenula (LHb) neurons are activated by aversive stimuli and their predictors. We recently showed also that LHb neurons show a biphasic response to cocaine, with an initial inhibition followed by delayed excitation that parallels the rewarding and aversive effects of cocaine. We sought to test whether these patterns of responses are also present in the entopeduncular nucleus (EPN), which provides a major afferent to the LHb. We found using retrograde labeling and cFos that rostral parvalbumine negative EPN neurons projecting to the LHb were activated by 30x footshocks and by 0.75 mg/kg intravenous cocaine infusion. Furthermore, the animals were conditioned with three different auditory cues: an appetitive cue paired with food pellet delivery, an aversive cue paired with a footshock, and a neutral cue paired with neither food nor footshock. Electrophysiological recordings from awake behaving animals further showed that most of neurons in the rostral EPN showed excitations to the footshock, and that cue-responsive EPN neurons exhibited relatively higher firing after aversive and neutral cues than after appetitive cues. Further experiments are underway to determine whether these neurons encode motivational properties of cocaine.

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140.12/H2. ***role for the lateral habenula and downstream efferent pathways in repeated probabilistic reversal learning***

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number of neuropsychiatric disorders including depression, autism spectrum disorder, and Parkinson's disease are characterized by deficits in the ability to rapidly switch behaviors under changing reward

contingencies. This ability to change responses when outcomes change is an executive function commonly termed cognitive flexibility. A common task used to test cognitive flexibility across species is known as reversal learning. Previous studies have shown that manipulations of serotonin (5-HT) and dopamine (DA) affect cognitive flexibility in tasks such as reversal learning. Importantly, these two neurotransmitters are known to play a role in a variety of neuropsychiatric conditions, including the ones mentioned, raising the possibility that a common mechanism may play a role across diseases. The lateral habenula (LHb) may be a key structure in mediating reversal learning as it is known to affect transmission of 5-HT and DA. Behaviorally, the LHb is thought to provide an error signal during decision making tasks making it likely that it is critically involved in tasks requiring learning from errors such as reversal learning. To test this hypothesis, a maze based probabilistic reversal learning task (PRL) with male Long-Evans rats was used to examine the role of the LHb via neurotransmitter inactivation with the gamma-aminobutyric acid (GABA) agonists baclofen and muscimol (50ng/0.2  $\mu$ L). Prior to behavioral testing rats were implanted with guide cannula aimed at the LHb for subsequent injections. The PRL task took place on a T-shaped maze with return arms. The correct arm resulted in reward on 80% of choices while the incorrect arm was never reinforced. Rats ran 200 trials per daily session. If an animal chose the correct arm over 10 consecutive trials, the reward contingences were reversed. Once animals were able to complete at least 3 reversals per session over consecutive days, injections began. Results revealed that inactivation of the LHb led to fewer overall reversals than rats injected with a saline control. Error analysis revealed a slight increase in perseverative errors and a large increase in regressive errors. Additionally, decreases in stay/win behavior and increases in shift/lose behavior indicated a generalized reward sensitivity impairment after LHb inactivation. Current work is utilizing designer receptors engineered and activated by designer drugs (DREADDs) to target LHb projections to dopamine systems to further understand its involvement in cognitive flexibility. Overall, these findings suggest that the LHb is important for learning and/or implementing switches in behavior when reward contingencies change.

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#### 141.27/H30. ***Impact of alcohol on human neural stem cells***

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Alcohol abuse causes an enormous impact on health, society, and the economy. Currently, there are very limited effective therapies available, largely due to the poor understanding of mechanisms underlying alcohol use disorders (AUDs). Neurogenesis from Neural Stem Cells (NSCs) is important in brain development and neuronal functions such as behavior, learning, and memory. Both neurons and NSCs are known to be sensitive to alcohol exposure, but most of the reported studies exploring effect of alcohol on NSCs are incomplete and often controversial. To better understand the effects of ethanol on NSC basal properties such as self-renewal and multipotency, we generated NSCs from induced pluripotent stem (iPS) cells (iPS-NSC) through epigenetic induction to the neural lineage and studied the neurotoxic effects of both acute or chronic exposure on both iPS cells and NSCs. We showed that transient exposure to a sub-lethal dose of ethanol (70mM, resembling alcohol blood concentration in

heavy drinkers) does exert a similar effect on iPS cells and NSCs. In particular, ethanol exposure for 24 hours or 7 days does not affect the proliferation or the multipotency of iPS cells and neural progenitors but primes an innate immune-like response by activating the inflammasome-mediated pathway. We observed a partial impairment of the mitochondrial and lysosomal patterns with a decrease of the number of iPS cell-derived neurons following ethanol exposure. However, the electrophysiological activity of alcohol challenged NSC-derived neurons is preserved, since they are able to fire action potentials similar to untreated cells. Our hypothesis is that ethanol exposure increases the vulnerability of NSCs to further damage (with specific reference to oxidative damage) that could originate either from genetic background (gene risk variants) or the external environment. Hence, unraveling the mechanisms underlying the neurogenic dysfunction induced by alcohol abuse will allow us to better predict how AUD patients will develop age related disorders. This will also elucidate how increased vulnerability of neural progenitors may contribute to the development of multifactorial disorders such as Alzheimer's Disease, Parkinson's Disease and lysosomal storage diseases.

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142.01/H33. ***Chronic intermittent nicotine delivery with lung alveolar region-targeted aerosol technology to rats produces circadian blood pharmacokinetics resembling human smokers***

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Cigarette smoke is an aerosol containing concentrated tiny particles that carry nicotine (Nic) into lung alveolar regions to be rapidly absorbed into the circulation. Episodic inhalation of Nic aerosol during smoking activates nicotinic receptors followed by a cycle of desensitization/resensitization. Smokers' exposure to Nic is a chronic intermittent process, with intake during wakefulness and abstinence during sleep (withdrawal) resulting in circadian fluctuation of blood Nic levels. Withdrawal plays an important role in Nic addiction which has been difficult to study properly due to lack of clinically relevant animal model that mimics episodic inhalation of Nic and produces circadian blood concentration pattern resembling that of human smokers. We previously developed a non-invasive method with alveolar region-targeted aerosol technology for delivering Nic to rodents that produced blood pharmacokinetics resembling smoking a cigarette in humans. We have now developed an integrated platform with computer control where freely moving rodents in a chamber are exposed to episodic Nic aerosol on scheduled intervals and number of times. We have optimized the parameters of aerosol generation and exposure. Nic aerosol was generated with a Collison nebulizer that contained a solution of 0.12% Nic in saline. Rats were exposed to Nic aerosol for 1 min every half hour, for a total of 24 times in each 12-hour (h) dark phase of 12/12 h dark/light circadian cycles for multiple days. Rats were returned to their home cage during the light phase when there was no aerosol delivery. We collected rat blood samples every 2 h at the end of the half hour intervals between aerosol deliveries during the 12-h dark phases and every 1 h during the light phases. Plasma concentrations of Nic and its metabolite cotinine were determined

with a LC-MS/MS method. Nic concentrations gradually increased during the dark phase and reached a plateau of 30 to 40 ng/ml in 8 to 10 from the start of the daily Nic aerosol deliveries. Nic levels gradually decreased to 5 to 10 ng/ml during the light phase when no aerosol was delivered. The circadian fluctuation patterns of blood Nic and cotinine levels match the circadian blood pharmacokinetics of human smokers (Benowitz et al., 1982). In summary, we have developed methods and devices that produce chronic intermittent Nic exposure animal models with the route of administration and circadian blood pharmacokinetics equivalent to those of human smokers. This methodology is a powerful tool for studies of behavior, pharmacology and toxicology of chronic Nic exposure, nicotine addiction, tobacco-related diseases, teratogenicity, and for discovery of therapeutics.

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142.03/H35. ***Alpha6-containing nicotinic acetylcholine receptors in midbrain dopamine neurons are poised to govern dopamine-mediated behaviors and synaptic plasticity***

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Acetylcholine acts through nicotinic and muscarinic acetylcholine (ACh) receptors in ventral midbrain and striatal areas to influence dopamine (DA) transmission. This cholinergic control of DA transmission is important for processes such as attention and motivated behavior, and is manipulated by nicotine in tobacco products. Identifying and characterizing the key ACh receptors involved in cholinergic control of DA transmission could lead to small molecule therapeutics for treating disorders involving attention, addiction, Parkinson's disease, and schizophrenia. Here, we used genetic, pharmacological, behavioral, and biophysical approaches to study nicotinic ACh receptors (nAChRs) containing the  $\alpha 6$  subunit.  $\alpha 6$ -containing nAChRs are highly and specifically expressed in midbrain DA neurons, making them an attractive drug target. For many experiments, we used mice expressing mutant  $\alpha 6$  nAChRs (" $\alpha 6L9S$ " mice) that increase the sensitivity of these receptors to agonists such as ACh and nicotine. Taking advantage of simple behavioral phenotype exhibited by  $\alpha 6L9S$  mice, we compared the ability of full versus partial  $\alpha 6^*$  nAChR agonists to activate  $\alpha 6^*$  nAChRs in vivo. Systemic administration of nicotine (0.02-0.05 mg/kg, i.p.) and the partial agonists varenicline (0.01-0.03 mg/kg, i.p.) and ABT-089 (1-3 mg/kg, i.p.) increased locomotor activity in  $\alpha 6L9S$  mice. Using local infusions of both agonists and antagonists into brain, we demonstrate that neurons and nAChRs in the midbrain are sufficient to account for this behavioral response. Intra-VTA infusion of nicotine (1.7 nmol) increased locomotor activity in  $\alpha 6L9S$  mice but not their wildtype counterparts, while co-infusion with the  $\alpha 6^*$  antagonist  $\alpha CtxMII$  (10 pmol) blocked the enhanced locomotor activating effects of nicotine in  $\alpha 6L9S$  mice. Ongoing studies are investigating the role of dopamine D1 receptors in the nucleus accumbens in the locomotor activation induced by nicotine in  $\alpha 6L9S$  mice. To complement these behavioral studies, we studied the ability of in vivo  $\alpha 6^*$  nAChR activation to support plasticity changes in midbrain DA neurons that are relevant to behavioral sensitization and addiction. By coupling local infusions of drugs and brain slice

patch clamp electrophysiology, we show that activating  $\alpha 6^*$  nAChRs in midbrain DA areas is sufficient to enhance glutamatergic transmission onto VTA DA neurons. Together, these results from in vivo studies suggest that  $\alpha 6^*$  nAChRs residing on VTA DA neurons are positioned to strongly influence both DA-mediated behaviors and the induction of synaptic plasticity by the addictive drug nicotine.

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142.04/H36. ***NRG3 modulates long-term synaptic plasticity in orbital frontal cortex***

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Cigarette smokers have difficulty quitting, which is thought to be related to deficient impulse control. Previous studies from our lab have shown that single-nucleotide polymorphisms across the gene for neuregulin 3 (NRG3) are linked to failed smoking cessation. recent preclinical study indicates that NRG3 in the frontal cortex regulates certain aspects of impulsivity. However, the mechanism by which NRG3 regulates neuronal signaling in this area, and hence impulsivity, is unknown. To begin to address this, we used electrophysiological field recording to assess whether NRG3 alters long-term potentiation (LTP) in orbital frontal cortex (OFC). We developed a protocol for LTP induction in OFC and found that NRG3 attenuated the expression of LTP. This NRG3-attenuated LTP was selectively rescued by Afatinib, an ErbB4 inhibitor. These data suggest that NRG3 may influence general impulsivity through modulation of long-term synaptic plasticity in the OFC. Current studies are evaluating the effect of chronic nicotine treatment on NRG3-mediated synaptic plasticity in the OFC. This study was supported by NIH/NIDA grants DA032681 (JRT) and DA031747 (PIO).

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142.06/H38. ***Does nicotine speed up subjective time or induce impulsivity?***

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Systemic nicotine induces premature responses when timing in the seconds-to-minutes range. It has been suggested that this effect reflects a nicotine-induced acceleration of the internal clock. An alternative explanation for this evidence posits that nicotine induces impulsive behavior by reducing the threshold for responses that result in more reinforcement. The clock-speed and response-threshold hypotheses were tested in rats in two experiments using a novel timing task. In this task, rats were trained to seek food at one location after 8 s since trial onset and at different location after 1 s. In Experiment 1, rats either received the same reward at both times (group SAME) or received larger reward at 16 s (group DIFF). In Experiment 2, rats either received a larger reward at 8 s (group SHORT) or received larger reward at 1 s (group LONG). Steady baseline performance was followed by days of subcutaneous nicotine administration (0.3 mg/kg), baseline recovery, and, in Experiment 1, an

antagonist challenge (mecamylamine, 1.0 mg/kg). Empirical and modeling analysis revealed that nicotine induced an immediate reduction in latencies to switch (LTS) between locations in all groups, but this effect was more prominent in groups DIFF and LONG than in groups SAME and SHORT. This effect was sustained throughout nicotine administration. Mecamylamine pretreatment and nicotine discontinuation rapidly recovered baseline performance. Additionally, the modeling analysis suggested that anomalous effects of nicotine on LTS dispersion may be due to a general loss of temporal control of behavior. Taken together, these results support the response-threshold hypothesis of nicotinic effects on timing, and suggest that the response threshold may be mediated by central nicotinic acetylcholine receptors.

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142.11/H43. ***Sex-differential effect on midbrain dopamine receptors of smoking***

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Evidence shows that female smokers have more difficulty in attaining long-term abstinence from cigarettes than male smokers. Although sex differences are important to consider when constructing a treatment plan, there still is a dearth of biological insight behind sex differences in smoking behavior. Previous studies show sex differences in dopaminergic transmission in the ventral striatum, the brain region through which nicotine produces rewarding effects. Given that, we hypothesize that cigarette smoking has a differential effect on D2-type receptors in the substantia nigra and ventral tegmental area (SN-VTA), which contains predominantly D2-short receptors having an inhibitory effect on dopamine release, between male and females. Twenty four smokers (14 men and 10 women) and 26 non-smokers (12 men and 14 women) participated. They were tested for dopamine D2-type receptor availability, indicated by binding potential (BPnd), in the SN-VTA using positron emission tomography (PET) with [<sup>18</sup>F] fallypride, a radiotracer with high affinity for D2-type receptors. In an exploratory analyses, other brain regions were tested by voxel-wise analysis and volume of interest analysis, depending on voxel significance. The interaction effect between sex and group (smokers vs. non-smokers) was significant, whereas main effects were not. The data revealed a trend of higher SN-VTA BPnd in female smokers than in female non-smokers, as well as a higher SN-VTA BPnd in female smokers than in males. This highlights the sex difference in dopamine D2-type receptors in the SN-VTA, suggesting it may be have relation to dopamine release in the striatum of smokers. In addition, voxel-wise analyses uncovered an interaction effect between sex and group on D2-type receptor availability in regions other than SN-VTA, such as amygdala, insula, temporal lobe, and striatum. These results support findings in previous studies and provide a platform to extend research in biological sex-differential effect of tobacco smoking.

142.13/H45. ***Subsecond modulation of dopamine release by the tobacco product flavorant menthol***

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Flavorants, such as menthol, are commonly added to tobacco cigarettes in order to increase appeal. Therefore, it is not surprising that menthol, and potentially other added flavorants, alters smoking behavior. Specifically, menthol smokers have their first cigarette of the day earlier compared to non-menthol, have a harder time quitting, and smoke fewer cigarettes per day. We postulate that these differences in smoking behavior arise from menthol's actions in the midbrain dopamine (DA) pathway, a critical circuit in the brain for mediating the reinforcing effects of natural rewards and drugs of abuse. Here, in male Sprague-Dawley rats, we demonstrate a novel approach for examining the interaction between flavorants and nicotine on nicotine taking and subsequent effects on phasic DA release. Combining intraoral delivery of flavorants (0.2mL/infusion) with fast scan cyclic voltammetry in freely-moving rats, we found that intraoral administration of sucrose (10%) and menthol (0.005%), but not water, elevates phasic DA release in the nucleus accumbens core in naïve rats. Each rat received water, menthol, and sucrose, in blocks of 25 infusions. The order of flavorant presentation was counterbalanced across rats. Moreover, there was no effect on flavorant presentation order nor within a block of infusions. In order to test the reinforcing value of each flavorant, we examined operant behavior for intraoral flavorant delivery using a FR-1 schedule of reinforcement. Rats readily self-administered intraoral sucrose but not menthol nor water. Ongoing experiments include combining intraoral flavorant and intravenous nicotine self-administration in order to examine the effects of flavorants on nicotine taking.

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142.18/I2. ***Functional upregulation of  $\alpha 4^*$  nicotinic acetylcholine receptors in VTA GABAergic neurons increases sensitivity to nicotine reward***

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Chronic nicotine exposure increases sensitivity to nicotine reward during a withdrawal period which may facilitate relapse in abstinent smokers, yet the molecular neuroadaptation(s) that contribute to this phenomenon are unknown. Interestingly, chronic nicotine use induces functional upregulation of nicotinic acetylcholine receptors (nAChRs) in the mesocorticolimbic reward pathway potentially linking upregulation to increased drug sensitivity. In the ventral tegmental area (VTA) functional upregulation of nAChRs containing the  $\alpha 4$  subunit ( $\alpha 4^*$  nAChRs) is restricted to GABAergic neurons. To test the hypothesis that increased functional expression of  $\alpha 4^*$  nAChRs in these neurons modulates nicotine

reward behaviors, we engineered a Cre recombinase-dependent gene expression system to selectively express  $\alpha 4$  nAChR subunits harboring “gain-of-function” mutation (a leucine mutated to a serine residue at the 9’ position: Leu9’Ser) in VTA GABAergic neurons of adult mice. In mice expressing Leu9’Ser  $\alpha 4$  nAChR subunits in VTA GABAergic neurons (Gad2VTA:Leu9’Ser mice), sub-reward threshold doses of nicotine were sufficient to selectively activate VTA GABAergic neurons and elicit acute hypolocomotion which developed tolerance with subsequent nicotine exposures compared to control animals. In the conditioned place preference procedure, nicotine was sufficient to condition a significant place preference in Gad2VTA: Leu9’Ser mice at low nicotine doses that failed to condition control animals. Together, these data indicate that functional upregulation of  $\alpha 4^*$  nAChRs in VTA GABAergic neurons confers increased sensitivity to nicotine reward and points to nAChR subtypes specifically expressed in GABAergic VTA neurons as molecular targets for smoking cessation therapeutics.

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142.19/13. ***Differential nicotine responses in human dopaminergic neurons derived from iPSC carrying CHRNA5 N398 variant***

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Many addictive drugs such as nicotine mediate reward and reinforcing mechanisms within the mesolimbic pathway involving midbrain dopaminergic (mDA) neurons via nicotinic acetylcholine receptors (nAChRs). Previously, genome-wide association analyses (GWAs) identified single nucleotide polymorphisms (SNPs) associated with increased risk of addictive phenotypes including a SNP encoding D398N (aspartate to asparagine) variation in the CHRNA5 gene encoding the alpha 5 subunit of nAChR. Since nicotine mediates reward within mDA neurons of the mesolimbic pathway, we differentiated iPSC to mDA cultures to test functional properties and response to nicotine. Patient-derived induced pluripotent stem cell (iPSC) lines were prepared from age- and gender-matched D398 and N398 variants with clinically defined nicotine use. We generated mature, nAChR-expressing, DA-releasing neurons using published methods. Gene expression and immunohistochemistry studies confirm that the majority of cells expressed standard mDA markers in neuronal cultures derived from iPSCs carrying either N398 or D398 variants. A minor fraction of cells expressed glutamatergic or GABAergic markers. The N398 variant differentially expresses lower levels of genes associated with glutamate, serotonin, and dopamine receptor signaling, suggesting an indirect effect on signaling. While both groups exhibited physiological properties consistent with neuronal function, the N398 neuronal population responded more actively to application of nicotine in electrophysiological analyses, consistent with reports of an enhanced initial response to nicotine in N398 subjects. Our results are consistent with altered N398 signaling of DAergic neurons, possibly due to effects in presynaptic glutamatergic cells present in our cultures.

142.23/17. ***Inhalation of aerosolized nicotine enhances cognitive function in aged mice using an automated aerosol delivery system***

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Tobacco use is the leading cause of preventable disease and disability in the U.S. Nicotine is the key chemical causing addiction to tobacco smoking. Current smoke cessation drugs produce about 20% long-term abstinence of smoking. Therefore, understanding nicotine pharmacology, reward effects and toxicology is critical to the development of more effective pharmacotherapies. However, animal models that mimic human nicotine kinetics, such as episodic inhalation and chronic intermittent exposure are lacking. We have developed an aerosolized nicotine delivery device integrated with our SmartCage technology. The system allows researchers to deliver a controllable amount of nicotine rapidly into the animal's systemic circulation. To test the system, we examined if nicotine aerosol inhalation in the amount similar to smoking cigarettes in human enhances learning and memory in aged mice. We administered either 0.5% aerosolized nicotine or saline to free moving mice (C57BL/6, male, 1 - 20 months old) in the aerosolized chamber for 5 min daily for consecutive 5 days. There were 91 ng/ml nicotine and 101 ng/ml cotinine in mouse plasma 5 min after the last exposure. The levels of nicotine and cotinine 3 min after exposure were 5 ng/ml and 22 ng/ml in mouse plasma, respectively, which resemble levels seen in heavy smokers. Thirty minutes after nicotine inhalation, mice were subject to the Morris Water Maze test. Probe finding was trained daily for consecutive 4 days and memory retention was assessed in the probe test. Inhalation of aerosolized nicotine did not produce a significant decrease time in finding the probe; but significantly enhanced memory in aged mice manifested by an increased time (21 s) in test quadrant compared to the aerosolized saline-control group (14 s) in test quadrant during the probe test. In the Y-maze and step through passive avoidance test, nicotine-treated mice showed modest but an insignificant increase in memory compared to saline-control. Furthermore, the system enables rodents to perform self-administration of aerosolized nicotine by either nose-poking or lever pressing. Initially food pellet reward accelerated the training of nose-poking or lever-pressing behavior. Optimization and evaluation of nicotine self-administration are underway. In summary, we show that nicotine aerosol inhalation in the amount similar to smoking cigarettes in human enhance memory in aged mice. The aerosol generation system can deliver a controllable amount of nicotine, and potentially other drugs of abuse, which induce behavioral changes, pharmacological or toxicological effects in preclinical setting similar to that occurs in human drug addicts.

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143.01/18. ***The role of the dynorphin-kappa-opioid system in reinstatement of nicotine-seeking in mouse self-administration***

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Nicotine is the most widely used addictive substance, and its use is accompanied by a high propensity for relapse. However, the neurobiological mechanisms underlying nicotine relapse/reinstatement remain unclear. Prior studies have shown that in rodents, activation of the kappa-opioid receptor (KOR) system via stress-induced dynorphin release elicits negative affective states, and thereby triggering reinstatement of drug-seeking behaviors. Therefore, our goal is to establish the role of the dynorphin/KOR system in stress-induced reinstatement of nicotine-seeking. We first trained male C57BL/6 mice to self-administer nicotine intravenously (0.03 mg/kg/infusion, 6 min sessions) on a fixed ratio-5 schedule of reinforcement for a minimum of 10 days. After stable levels of nicotine intake were established, mice underwent extinction training until criterion was reached ( $\leq 20\%$  of responding compared to last nicotine self-administration session). We then investigated whether activation of KORs were sufficient to induce reinstatement of nicotine-seeking. Indeed, mice showed a robust reinstatement response after administration of the KOR agonist, U50,488 (2.5-5.0 mg/kg, i.p., 3 min prior to reinstatement test). This data suggests that activation of the KOR system is sufficient to cause nicotine-seeking in mice. Currently, we are exploring whether KORs are necessary for stress-induced reinstatement of nicotine-seeking via administration of systemic KOR selective antagonists. Future follow-up studies will focus on dissecting the specific neural circuitry underlying KOR-mediated reinstatement of nicotine-seeking.

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143.02/19. ***Partial inhibition of monoamine oxidase (MAO) increases nicotine self-administration in rats***

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Monoamine oxidase (MAO) is inhibited in the brains of chronic cigarette smokers by approximately 40%. While some compounds in cigarette smoke that inhibit MAO have been identified, it is not clear to what extent these compounds are responsible for the MAO inhibition seen in smokers. Prior studies examining the significance of the MAO inhibition to smoking behavior have used known MAO inhibitors to examine their effect on nicotine self-administration in rats. These studies have shown that MAO inhibitors can enhance responding for intravenous (i.v.) infusions of nicotine, especially at low doses of nicotine. However, these studies have relied on large doses of MAO inhibitors that produce near complete inhibition of MAO, and in some studies the doses are large enough to produce substantial off-target effects. The goal of the present study was to examine the effects of doses of tranylcypromine (TCP) that produce partial inhibition of MAO on i.v. nicotine self-administration in adult rats. Rats responded for a low dose of nicotine (10  $\mu$ g/kg/infusion) in daily 1-hr sessions and received an i.p. injection of 0, 0.1, 0.3, or 1.0 mg/kg of TCP 1-hr prior to each self-administration session. TCP produced dose-dependent increase in the rate at which rats acquired stable nicotine self-administration, as well

as the rate of nicotine self-administration during stable maintenance. The average number of infusions earned during the final three days of self-administration on a fixed-ratio 2 schedule of reinforcement was 7.75 in the saline group (SEM=1.59, n=21). Rats receiving 0.3 mg/kg TCP earned an average of 18.48 infusions (SEM=2.08, n=21), and MAO activity, measured in the dorsal striatum from a subset of brains collected at the end of the final session, was 20% of that in control animals. Across all doses of TCP, there was a significant correlation between nicotine self-administration and MAO inhibition. These data suggest that in the range of partial MAO inhibition seen in cigarette smokers, MAO inhibition increases responding for nicotine. Therefore MAO inhibition caused by cigarette smoking may interact with nicotine to promote smoking behavior, and this may be particularly relevant at low doses of nicotine. These data may be important for the FDA as they consider a mandated reduction in the nicotine content of combustible products. These data suggest that cigarette constituents that inhibit MAO are likely to shift the reinforcing value of low-nicotine products.

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#### 143.04/111. *Histamine and nicotine self-administration*

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Histamine is a neurotransmitter in the brain as well as a modulator in the periphery. As such, it does more than control secretions in the stomach and sinuses. Histaminergic neural circuits play important roles in a variety of behavioral functions. Histamine H1 receptors have been found in our earlier studies and others' to play critical roles in a variety of behavioral functions from sensorimotor modulation, to cognition to maintenance of reinforced behavior. Concerning reinforced behavior, we found that acute or chronic dosing of the histamine H1 antagonist pyrilamine significantly reduced nicotine self-administration in rats. H1 antagonist treatment may be useful as an aid for smoking cessation. To further the characterization of histaminergic involvement in nicotine self-administration, we investigated the effects of an H1 histamine receptor agonist, betahistine. Female young adult Sprague-Dawley rats were trained to self-administer nicotine (FR1, 0.03 mg/kg/infusion) in one-hour sessions. In the first study, betahistine (0, 2, 4, 8 and 16 mg/kg) was acutely administered to determine the dose-effect range for potentially increasing nicotine self-administration. Trends were found that 8 mg/kg of betahistine increased nicotine self-administration. This dose was not found to affect locomotor activity. In the second study a different set of rats were trained in the same way to self-administer nicotine. The rats were divided into two groups matched for baseline nicotine self-administration. One group was given 8 mg/kg of betahistine before every subsequent nicotine self-administration session (N=17), while the other group was given control saline injections (N=19). Chronic betahistine significantly (p<0.05) escalated increasing nicotine self-administration. This further shows that histamine plays important roles in the level of nicotine self-administration, particularly further elevating higher levels of nicotine self-administration. Histaminergic system involvement in nicotine self-administration may be related to stress effects increasing tobacco addiction.

143.05/112. ***Imaging CA1-hippocampal neuronal ensembles during nicotine-dependent contextual associations***

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Learned associations between environmental cues and the rewarding properties of addictive drugs are a major cause for relapse among drug addicts. The hippocampus (HIP) is therefore a likely key player in the development of addictive behaviors. The link between learning and memory systems, contextual cues, and reward circuitry is largely unknown. The conditioned place preference (CPP) paradigm attempts to model this aspect of drug reward-associations and can be useful in examining the underlying neural circuitry involved in the formation of drug-associated memories. Here we combine in vivo calcium imaging of CaMKII $\alpha$  CA1 HIP neurons with CPP to study the role of the HIP in nicotine-induced behaviors. We injected AAV5-CaMKII $\alpha$ -GcAMP6f-eYFP into the HIP CA1 area and, implanted 1 mm diameter and 4 mm length GRIN lens is 100 microns above the injected region. After another 1-2 weeks, we use a microscope camera from INSCOPIX to observe this the neuron activity in the CA1 area can be observed from the microscope. We then used a standard unbiased, counterbalanced CPP protocol to observe neuronal activity during the development of nicotine CPP. Mice were pre-tested in the CPP boxes on day 1, and days 2-3, they received saline in the AM and nicotine (0.5mg/kg, s.c.) in the PM for 2 min session. On day 5, they were tested for nicotine place preference, as determined by the time they spent in the drug-paired chamber post-test minus pre-test. After collecting all data from each session, Mosaic™ is used to preprocess the data by reducing dataset, meaning filter, motion correction. By PCA/ICA, single neuron activity was separated and sorted manually. Each session retrieved one dataset for single neuron spatial filters and one dataset set for their time course. After comparing neuron activity between two CPP chamber within posttest section, we found distinct patterns of neuronal activity when a mouse enters the nicotine-paired chamber compared to the saline-paired chamber potentially indicating that cue-reward neuronal activity is potentiated during conditioning and formation of preference.. Follow-up studies were conducted in which AAV5-CaMKII $\alpha$ -HA-hM4D(Gi)-IRES-mCitrine was injected into the CA1 HIP region to show that silencing CA1, CAMKII+ cell activity in this is sufficient to block the development of nicotine-induced CPP. Taken together, our data provide unique evidence for key role of the CA1 HIP region in nicotine-contextual associations and begin to dissect the circuitry mediating the development of drug-reward cue associations.

**143.06/113. Nicotine self-administration is enhanced in obesity-resistant rats**

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Cigarette smoking and obesity represent the largest challenges to public health. Smokers with higher body mass index (BMI) smoke more cigarettes per day and may be more nicotine dependent than lean smokers. However, very little is understood about the relationship between obesity and nicotine reinforcement. Furthermore, the FDA is considering a policy of markedly reducing the allowable nicotine content in cigarettes; obese smokers may respond differently to nicotine reduction in cigarettes. To model obese smokers, adult male Sprague-Dawley rats (Charles River; Kingston facility) were maintained on a high fat diet (HFD; 31.8 kcal from fat) for two weeks. Body weight gain distributed into distinct tertiles: diet-induced obese (DIO), DIO-resistant (DR), and a middle group, which was maintained on chow for the remainder of the experiment, as a diet control (Chow). Rats learned to self-administer 60 µg/kg/infusion nicotine on a fixed-ratio (FR) 2 for ten days before the schedule was escalated to FR5. To establish a nicotine dose response curve, and to model nicotine reduction policy, nicotine dose was halved every seven days to reach 3.75 µg/kg/infusion, a dose previously reported to be sub-threshold for nicotine reinforcement in rats eating a restricted standard chow diet. The Chow group took more nicotine than DR and DIO rats at 60 µg/kg/infusion, although there were no differences in the frequency or proportion to acquire self-administration behavior across groups. Together, these data indicate that at a population level, there are no differences in the probability that rats will acquire self-administration, but that Chow rats respond at higher rates, at least for high doses of nicotine. At all other doses, however, the dose response curve for the DR group was shifted upwards, such that the rats took more infusions than the DIO group ( $p < 0.05$ ). The increase in infusions in the DR group was not explained by increased activity, as measured by inactive responding. The differences between groups are also not explained by differences in body weight, as there is no relationship between nicotine consumption or infusions earned and body weight. Together, nicotine self-administration, particularly at moderate and low doses, is enhanced in rats resistant to diet-induced obesity. These data indicate that current lean smokers eating a densely caloric diet may continue to smoke at high rates following the reduction of nicotine in cigarettes, prolonging the exposure to the harmful chemicals in cigarette smoke.

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**143.07/114. Activation of the kappa-opioid receptor system is both necessary and sufficient for reinstatement of nicotine place preference**

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The Kappa-opioid receptor (KOR) system plays a conserved role in stress-induced behavioral responses including reinstatement of drug seeking behavior for nearly every major drug of abuse. Due to nicotine's high propensity for stress-induced relapse, we hypothesized that stress would also induce reinstatement of nicotine seeking behaviors in a KOR-dependent manner. We used a standard unbiased, counterbalanced conditioned place preference (CPP)/reinstatement protocol in mice to investigate the role of KORs in mediating stress-induced behavioral responses to nicotine. Mice were pre-tested in the CPP boxes on day 1, and on days 2-3 mice were conditioned, receiving saline in the AM and nicotine (0.5mg/kg, i.p.) in the PM for 20 min sessions. On day 5, mice were tested for nicotine CPP, determined by the time they spent in the drug-paired chamber post-test minus pre-test. Animals were extinguished for two days using saline pairings, prior to the reinstatement phase. We found that the widely used pharmacological stressor Yohimbine (Yoh) (2mg/kg, i.p.) 5 minutes prior to reinstatement post-testing causes reinstatement of nicotine CPP. This reinstatement of nicotine CPP is NorBNI sensitive, indicating that KOR activity is necessary for Yoh-induced nicotine CPP reinstatement. To determine if KOR activation alone is sufficient for reinstatement of nicotine CPP, we injected the KOR agonist U50,488 (5mg/kg) 30 minutes prior to reinstatement, and found that KOR activation was sufficient to reinstate nicotine place preference. Two hours following the reinstatement test, mice were perfused to examine the effects on Yoh on neuronal activation (c-fos) in the presence and absence of KOR signaling. We visualized robust c-fos expression in the Basolateral Amygdala (BLA) following Yoh treatment, which was significantly reduced in mice pre-treated with norBNI prior to Yoh-exposure. Follow-up studies were conducted to locally inactivate KOR or neuronal activity in the BLA, to assess the influence of KOR-expressing cells and neural circuits on nicotine CPP. NorBNI injected locally into the BLA blocked Yoh-induced nicotine-CPP reinstatement without affecting the acquisition of nicotine CPP. In a separate experiment, we found that activation of hM4D(Gi) DREADDs in the BLA by CNO is sufficient to reinstate nicotine CPP. These data suggest a role for BLA KORs in stress-induced nicotine seeking. Future studies will attempt to further dissect this BLA circuitry to identify cell type-specificity of these KOR circuits involved with stress-induced reinstatement of nicotine CPP.

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143.08/115. ***Trans-generational effects of paternal nicotine self-administration***

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Recent evidence indicates that paternal smoking is associated with nicotine dependence and increased incidence of childhood cancer in offspring. These findings indicate that voluntary nicotine taking influences behavioral phenotypes in future generations. The goal of this study is to establish a novel rodent model in which nicotine-experienced sires confer enhanced/increased vulnerability to nicotine reinforcement in their offspring and grand offspring. Male Sprague Dawley rats were allowed to self-administer nicotine (0.03 mg/kg/infusion) on a fixed-ratio 1 (FR1) schedule of reinforcement for 60 consecutive days. Each nicotine-experienced rat was paired with a yoked saline control rat that received the same number and temporal pattern of infusions. Following nicotine self-administration, nicotine-

experienced and yoked saline control rats were allowed to mate with drug-naïve dams. When the offspring (F1) reached 60 days of age, acquisition and maintenance of nicotine self-administration were assessed. Both female and male nicotine-sired offspring self-administered significantly more nicotine than saline-sired offspring. Based on these results, drug-naïve, nicotine- and saline-sired male and female F1 littermates were bred with drug-naïve counterparts in order to produce an F2 generation (grand offspring of nicotine- and saline-experienced sires). Acquisition and maintenance of nicotine self-administration was then assessed in the male and female grand-offspring. Our preliminary results indicate that F2 males of nicotine-sired F1 females had a propensity to self-administer more nicotine than controls. Taken together, these data are consistent with human epidemiological studies and indicate that voluntary paternal nicotine taking increases susceptibility to nicotine taking in subsequent generations. Identifying novel epigenetic mechanisms underlying the transmission of enhanced vulnerability to nicotine dependence will aid in the development of novel smoking cessation medication in generations at high risk for chronic smoking behavior.

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143.09/116. ***Azacytidine regulates socially-acquired nicotine intravenous self-administration (IVSA) in rats***

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Social environment is a critical factor in cigarette smoking. Previous studies demonstrated that social learning reversed conditioned flavor aversion (CFA) induced by self-administered nicotine in rats. We first determined the effect of 5-Azacytidine (5-Azac), a DNA methyltransferase inhibitor, on socially-acquired nicotine IVSA. Carbon disulfide (CS<sub>2</sub>), a component of the rodent breath, was used as a surrogate for the demonstrator rat to mediate social learning as reported. Licking on the active drinking spout in operant chambers meeting a fixed-ratio 10 reinforcement schedule resulted in the concurrent delivery of nicotine (i.v.) and an olfactogustatory (OG) cue containing CS<sub>2</sub> (0 or 500 ppm), 0.4% saccharin and 0.1% unsweetened grape KoolAid. CS<sub>2</sub> did not enhance the acquisition of nicotine SA in the three daily training sessions. However, CS<sub>2</sub> significantly increased the number of active licks on CFA tested on day four (OG + CS<sub>2</sub> vs OG: p<0.05). However, it significantly diminished the effect of CS<sub>2</sub> (5-Azac vs aCSF, p<0.01). In saline rats, CS<sub>2</sub> enhanced the operant response during both the acquisition and test sessions. This effect was inhibited by 5-Azac (i.c.v., training: p<0.01; testing: p<0.05). Similarly, bilateral infusions of 5-Azac (5 ng/ul, 0.2 ul/side) into the infralimbic cortex (IL) reduced the number of active licks on the CFA test (OG+CS<sub>2</sub> vs OG, p<0.01). In contrast, a low dose of 5-Azac (1 ng/ul, 0.2 ul/side) elevated the number of active licks on the first day of IVSA training (aCSF vs 5-Azac: p<0.01) and on CFA test (aCSF vs 5-Azac: p<0.001) in rats received OG+CS<sub>2</sub>. These data indicated that the effect of 5-Azac on social transmission of nicotine preference is region and dose specific. While i.c.v. administration and high dose IL administration reduced social learning, low dose application limited to the IL enhanced social learning. Thus, DNA methylation in IL is an important mechanism via which social learning promotes voluntary nicotine intake.

143.11/118. ***Genetic factors contribute to nicotine intravenous self-administration (IVSA) with menthol cue and sensation seeking***

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Menthol is the most widely used tobacco additive and is preferred by ~25% of US smokers. We previously reported a rat model of nicotine IVSA with orally delivered menthol as a sensory cue (Front Behav Neurosci 2014). We found that although taste and odor were associated with the aversive effect of nicotine, the cooling sensation of menthol was a conditioned cue for the reinforcing effect of nicotine. Genetic factors contributing to the preference for menthol-flavored tobacco products were difficult to ascertain from human studies, partly because of targeted advertisement in the minority populations. We studied the amount of nicotine intake with menthol cue in eight inbred strains of rats. These strains included the Brown Norway, Buffalo, Copenhagen, Dahl salt sensitive, Fisher 344, Lewis, Spontaneous hypertensive rat, and Wistar-Kyoto. All rats were implanted with jugular catheter on approx. postnatal day 38. Daily 2.5 h nicotine IVSA sessions were conducted in operant chambers equipped with two lickometers. Licking on the active spout completing a fixed-ratio 10 schedule triggered the concurrent delivery of 60  $\mu$ l menthol solution (0.01%) to the spout and iv nicotine (30  $\mu$ g/kg). No audio or visual cue was used. Rats were not food or water deprived. Nicotine intake was highest in the Dahl salt sensitive strain ( $9.6 \pm 1.1$  infusions per session) and lowest in the Buffalo strain ( $0.55 \pm 0.22$  infusions per session) during the last 3 sessions. The estimated heritability ( $h^2$ ) was 0.68. Each rat was also trained in an operant sensation seeking (OSS) protocol. These 1 h sessions were conducted in operant chambers fitted with two nose poke holes. Activating the active hole (fixed-ratio 2) turned on one of the two randomly chosen cue lights at a random frequency (0.5, 1, 2, 4 Hz) for a random duration (2, 4, 6, 8 s). Heritability for the number of rewards earned during the last three OSS sessions was 0.87. There was moderate correlation between nicotine intake with menthol cue and reward earned during OSS sessions (Pearson = 0.53,  $p = 0.18$ ). These data suggested that both nicotine intake with menthol cue and sensation seeking are heritable, and that sensation seeking is likely contributing to voluntary nicotine intake with menthol cue.

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143.12/119. ***Hypothalamic involvement nicotine self-administration in rats***

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The hypothalamus is brain region that has typically been overlooked regarding its potential contributions to processes of drug addiction. Classic literature has shown hypothalamic involvement in consummatory behavior. Drug addiction shares with feeding regulation key behavioral aspects of the appetitive urge. In addition to its primary role as a regulator of metabolic and autonomic function, the hypothalamus is a limbic structure composed of several distinct nuclei that both project to and receive

input from several regions of the brain, including areas involved in memory, attention, emotion, and reinforcement learning. The functional output of these projections is responsive to, and regulated by, dopaminergic, serotonergic, noradrenergic, and cholinergic activity. We are investigating the contributions of hypothalamic nuclei to nicotine addiction in a rat model of nicotine self-administration. The first series of experiments targeted D1 dopamine receptors located in the supramammillary nucleus (SuM) of the hypothalamus; a region that has been implicated in the process of positive reinforcement. Young adult female Sprague-Dawley rats were fitted with jugular catheters and given access to self-administer nicotine (0.03 mg/kg) on an FR1 schedule of reinforcement. Each self-administration session lasted 45 min. Bilateral infusion cannulae were implanted in the SuM to allow local infusion of the D1 receptor antagonist SCH23390. Infusions of SCH23390 occurred 5 min prior to the start of each session. Doses of SCH23390 (1, 2, and 4 µg/side) were infused in a repeated measures, counterbalanced design two times for each rat. Bilateral infusions of SCH23390 into the SuM caused significant reductions in the number of nicotine infusions per session at the 2 ( $p < 0.05$ ) and 4 ( $p < 0.01$ ) µg/side doses when compared to infusions of the aCSF vehicle. These results demonstrate that hypothalamic D1 dopaminergic innervation plays an important role in the process of nicotine self-administration. Hypothalamic mechanisms may be key components of the neural circuitry underlying addiction. Future experiments will examine the contributions of other transmitters innervating hypothalamic nuclei to nicotine addiction including the involvement of serotonergic and cholinergic receptor mechanisms to this process.

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143.14/121. ***Effects of menthol on nicotine-taking and -seeking behavior in rat models of nicotine dependence: implications for tobacco product control policy making***

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Tobacco smoking is a leading preventable cause of premature death in the US. Menthol is a significant additive in tobacco products. Clinical evidence suggests that menthol may promote tobacco smoking and nicotine dependence. However, it is not clear whether menthol enhances the reinforcing actions of nicotine and thus facilitates nicotine consumption. Investigating how menthol influences nicotine-addictive behavior may provide important information for making policies to regulate tobacco products. This study employed rat models of nicotine use to examine effects of menthol on nicotine-taking and -seeking behavior. Male Sprague-Dawley rats were trained in daily 1-h sessions to press a lever for intravenous nicotine self-administration under a fixed-ratio 5 schedule. A nicotine-conditioned cue was established via association of a neutral sensory stimulus with each nicotine infusion. In the subsequent extinction sessions, responding was extinguished by withholding nicotine delivery. On the following day after extinction, reinstatement tests were performed. Menthol administration (5 mg/kg, intraperitoneal or intraoral) was given 5 min prior to sessions. The preliminary results showed that menthol increased self-administration of nicotine at 0.015 mg/kg/infusion, a dose on the ascending limb of the inverted “U” shaped nicotine dose-response curve. Continued administration of menthol sustained active lever

responses in the extinction sessions. Moreover, re-administration of menthol after extinction effectively reinstated active lever responses. These data demonstrate a facilitative effect of menthol on nicotine-taking and -seeking behavior, suggesting that menthol in tobacco products may promote consumption of nicotine and contribute to the perseverance of tobacco-seeking behavior.

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143.17/124. ***Role for Tcf712 in regulating nicotinic acetylcholine receptor function and nicotine intake***

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Tobacco smoking is a major cause of premature death and disease, resulting in a significant economic burden on the United States healthcare system. Nicotine is the major psychoactive component of tobacco responsible for sustaining the tobacco smoking habit. Nicotine acts in the brain by stimulating neuronal nicotinic acetylcholine receptors (nAChRs). The positive reinforcing effects of nicotine are related to activation of high-affinity  $\alpha 4\beta 2$  nAChRs in the midbrain dopamine system. Conversely, aversive effects of nicotine are regulated by nAChRs containing  $\alpha 5$ ,  $\alpha 3$  and/or  $\beta 4$  subunits in medial habenula (MHb) neurons that project to interpeduncular nucleus (IPN), with these subunits highly enriched in the MHb-IPN circuit. The molecular mechanisms that restrict  $\alpha 5$ ,  $\alpha 3$  and/or  $\beta 4$  subunit expression to the MHb-IPN system, and the thereby control nicotine intake, are unknown. Here, we report that components of the Wnt signaling cascade, including Wnt glycoproteins, Fzd receptors and Tcf712, show remarkable enrichment in the MHb-IPN pathway in adult brain. We find that Wnt signaling is constitutively active in MHb neurons. Genetic disruption of Wnt signaling in the brains of rats, accomplished by genetic deletion of the Wnt transcription factor Tcf712, decreased  $\alpha 5$  subunit gene expression in the MHb. Moreover, we found that nAChR-mediated transmission in the MHb and IPN were disrupted in the Tcf712 knockout rats, measured using <sup>86</sup>Rubidium [<sup>86</sup>Rb<sup>+</sup>] efflux from synaptoneurosomes. As nAChR transmission in MHb and IPN is known to depend largely on nAChRs that contain  $\alpha 5$  subunits, these data further support a role for Wnt signaling in regulating nAChR function in the MHb-IPN system. Finally, we found that Tcf712 knockout rats consumed significantly more nicotine than their wildtype counterparts. Together, these data identify a key role for Wnt signaling, mediated through the transcription factor TCF712, in regulating the function of  $\alpha 5$  subunit-containing nAChRs in the MHb-IPN system. Moreover, Wnt signaling in this system appears to play a key role in controlling the motivational properties of nicotine. \*The first two authors contributed equally to this work

143.24/131. ***Inhibition of aldehyde dehydrogenase-2 (ALDH-2) suppresses nicotine self-administration in rats***

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ALDH-2 inhibitors have been shown to reduce cocaine and alcohol self-administration in rats by reducing drug-induced dopamine (DA) production in the VTA and DA release in the nucleus accumbens (Arolfo et al, 2009; Yao et al., 2010). The main goal of this study was to explore the potential of selective ALDH-2 inhibitor for reducing nicotine self-administration. Rats were trained to self-administer nicotine intravenously (iv) via their jugular vein. After acquiring a stable baseline for nicotine self-administration, rats were given an oral administration of one of the three doses (5, 10 or 30 mg eq/kg, calculated based on parent drug) of the pro-drug GS-6637 or vehicle 1 hr before nicotine self-administration session. Results showed that the acute administration of GS-6637 at 1 and 3 mg eq/kg significantly reduced nicotine-self-administration when compared with vehicle treatment (46% and 67%, respectively). Similarly, chronic oral administration of GS-6637 for consecutive days showed significant effect by reducing nicotine self-administration at 1 and 3 mg eq/kg (39% and 6 % inhibition, respectively) without the development of tolerance. In order to make direct comparison with varenicline (Chantix®), one of the few therapies approved as an aid to smoking cessation, a separate group of animals was administered single doses of varenicline at 1.6, 3.2 and 6.4 mg/kg. Consistent with previous reports (Rollema et al., 2007), significant inhibitions of nicotine self-administration was observed at the 3.2 and 6.4 mg/kg doses (52% and 49%, respectively). In conclusion, GS-6637 administered orally either acutely or chronically, can reduce nicotine self-administration without development of tolerance suggesting its promise as an aid for smoking cessation (Supported in part by NIDA P50 DA027840 and Gilead Sciences Inc.).

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143.26/133. ***Nicotine reinforcement and relapse are prevented by pharmacological enhancement of kynurenic acid in rats and squirrel monkeys***

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Tobacco smoking remains one of the leading causes of illness and death in the United States. Current anti-smoking medications, such as bupropion and varenicline, have limited effectiveness and are associated with high rates of relapse. Therefore, there is pressing need for newer, more effective treatment strategies. Recently, we demonstrated that enhancing brain levels of kynurenic acid (KYNA)\_which is an endogenous neuroinhibitory product of tryptophan metabolism and an allosteric modulator of alpha-7 nicotinic receptors\_selectively counteracts the abuse-related behavioral and

neurochemical effects of cannabinoids. However, there have been no studies examining whether increasing endogenous levels of KYNA can decrease nicotine reinforcement and relapse to nicotine seeking after a period of abstinence. In the present study, we enhanced KYNA levels by administering the kynurenine 3-monooxygenase (KMO) inhibitor Ro 61-8048. We investigated the effects of this treatment on: (1) nicotine self-administration in squirrel monkeys and rats; (2) drug-induced and cue-induced relapse to nicotine-seeking behavior in abstinent rats and monkeys; and (3) nicotine-induced elevation of dopamine levels in the nucleus accumbens shell (NAcS) of freely-moving rats. In these experiments, enhancing endogenous KYNA levels blocked nicotine self-administration and attenuated nicotine-induced dopamine release in the NAcS. Moreover, it prevented relapse-like effects induced by re-exposure to either nicotine or cues that had previously been associated with nicotine. These findings suggest that KMO inhibition should be further investigated as a promising new approach for the treatment of nicotine addiction.

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143.29/136. ***Effect of  $\alpha 4\beta 2$  and  $\alpha 3\beta 4$  nAChR-directed compounds on nicotine and alcohol co-addiction***

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Alcohol and nicotine addiction are highly co-morbid, indicating overlapping neural substrates. Recently, considerable interest has been attributed to the habenular/interpeduncular (MHb/IPN) cholinergic pathway, thought to play a role in nicotine addiction as well as addiction to other drugs of abuse. The MHb and IPN highly express nicotinic acetylcholine receptors (nAChR), particularly the  $\alpha 3\beta 4$  nAChR subtype, which is thought to be implicated in both nicotine and alcohol taking behaviors. Also, it has been proposed that the smoking cessation medication varenicline, commonly considered a  $\alpha 4\beta 2$  nAChR partial agonist, blocks alcohol self-administration by stimulating  $\alpha 3\beta 4$  nAChR. We examined the effect of  $\alpha 3\beta 4$  and  $\alpha 4\beta 2$  nAChR-directed compounds in an operant co-administration procedure in which rats concurrently self-administer nicotine (30  $\mu\text{g}/\text{kg}$  through jugular catheter) and alcohol (10% orally). The tested compounds were the  $\alpha 3\beta 4$  partial agonist AT-1001, the  $\alpha 4\beta 2$  partial agonist varenicline, TPI-202, selective  $\alpha 4\beta 2$  antagonist; and TPI-2212-59, selective  $\alpha 3\beta 4$  antagonist. Results demonstrated that the two  $\alpha 3\beta 4$  nAChR compounds, AT-1001 and TPI-2212-59, both attenuated nicotine self-administration at doses that did not alter alcohol self-administration whereas the  $\alpha 4\beta 2$  ligands, varenicline and TPI-202, both attenuated the self-administration of both reinforcers. When alcohol was used as the only reinforcer, AT-1001 was found to affect self-administration only at doses that also reduced food-maintained responding. However, a dose of AT-1001, which had no effect on alcohol or food self-administration (1.5 mg/kg), essentially eliminated reinstatement of alcohol seeking induced by yohimbine (0.625 mg/kg) whereas, reinstatement induced by alcohol-associated cues was not altered, nor did AT-1001 induce reinstatement of extinguished self-administration on its own. These data suggest that partial activation/functional inhibition at  $\alpha 3\beta 4$  receptors would primarily act on decreasing nicotine-reinforced behavior, while targeting  $\alpha 4\beta 2$  appears to modulate both alcohol and nicotine taking. However,  $\alpha 3\beta 4$  nAChR plays a role in mediating stress-related alcohol disorders.

143.30/137. ***Behavioral and neural effects of cigarette craving regulation using a proximal/distal reappraisal strategy in young-adult smokers***

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Tobacco smoking is a leading preventable contributor to death and disease with an estimate of over 500,000 smoking-related, premature deaths in the U.S. alone. Craving for nicotine-containing products, such as cigarettes, is a major factor in the maintenance of use. Behavioral strategies to help with regulation of craving may be helpful for reducing cigarette smoking, yet remain underexplored. The current fMRI study uses behavioral and brain measures to examine craving regulation in young smokers. Fifteen, young-adult cigarette smokers (11 males, 18-25 years old), who abstained overnight from smoking, underwent fMRI scanning before and after smoking a cigarette. We adapted a regulation strategy, often used in emotion regulation studies and based on proximal/distal self-positioning, to the context of cigarette craving. Prior to viewing video clips of people either smoking cigarettes (smoke) or not smoking (non-smoke), participants were instructed to either imagine themselves immersed in the scene, allowing themselves to experience any sensations that arose ("close", i.e., reactivity), or to imagine themselves at a distance from the scene ("far", i.e., regulation), making factual, objective observations of the content of the scene (e.g., indoors/outdoors). Participants rated their craving after each video. Behavioral results indicated main effects of smoking, proximity (close/far), and video type (smoke/nonsmoke videos) on cue-elicited craving [[unable to display character: &#8211;]] lower craving ratings were given after smoking, following the "far" versus "close" instructions, and viewing the non-smoke versus smoke videos. For the far-versus-close contrast (index of regulation), fMRI activation was greater after smoking a cigarette in brain regions associated with cognitive control, including right inferior frontal gyrus, bilateral anterior insula, anterior cingulate, bilateral putamen, and bilateral posterior parietal cortex. These preliminary results suggest that reappraisal strategies have an impact on cue-elicited self-reported craving, and that smoking a cigarette after a period of abstinence may increase capacity for neural processes related to cognitive control that are important for craving regulation.

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144.01/138. ***D1 and D2 receptors in the infralimbic and medial orbitofrontal cortices differentially mediate the reinstatement of cocaine seeking in rats***

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Prior work indicates that the medial prefrontal cortex (mPFC) plays a crucial role in mediating drug-seeking behaviors. Specifically, the infralimbic cortex (IL) within the mPFC has been shown to suppress cocaine-seeking behaviors. Despite the established dopaminergic innervation of this structure, the specific role that infralimbic dopamine plays in mediating the reinstatement of cocaine seeking is

unknown. Moreover, whether the medial orbitofrontal cortex (mOFC), a separate, more anterior region of the mPFC, regulates cocaine-seeking behaviors is not clear. Thus, the current set of experiments examined whether D1 or D2 receptor activation within the IL and the mOFC is involved in cocaine seeking during a variety of reinstatement tests. Male Sprague-Dawley rats underwent surgery for implantation of bilateral cannulas aimed at either the mOFC or the IL and insertion of an intravenous jugular catheter. After recovery, all animals underwent cocaine self-administration training for at least 1 days (2 h daily) followed by extinction training for a minimum of 7 days. After rats met extinction criteria, reinstatement testing began, which consisted of cued, cocaine-prime, and cue + cocaine-prime reinstatement tests. Immediately prior to reinstatement testing, rats received microinjections of the D1 antagonist SCH 23390, the D2 antagonist sulpiride, or their respective vehicles. Results indicated that D1 receptor blockade in the IL reduced cued reinstatement but had no effect on cocaine-prime and cue + cocaine-prime reinstatement. In contrast, D1 receptor blockade in the mOFC resulted in a blockade of all reinstatement types. Additionally, blocking D2 receptors in the mOFC had no effect on any reinstatement type. Ongoing experiments have found that D2 receptor blockade in the IL reduces cocaine-prime reinstatement. These findings suggest that D1 receptor activation in the mOFC is required for all types of reinstatement examined whereas, in the IL, such activation is involved in cued, but not cocaine-prime, reinstatement. Additionally, although D2 receptor blockade in the mOFC had no effect on cocaine-seeking behaviors, blocking these receptors in the IL appears to alter cocaine-prime reinstatement. Moreover, in contrast to previous work suggesting that IL activity is involved in suppressing cocaine seeking, our findings suggest that D1 and D2 receptor activation in the IL promotes cocaine seeking. Ongoing studies will further elucidate the role of D2 receptors in the IL to determine whether D1 and D2 receptors within the IL play discrete roles in mediating cocaine-seeking behaviors.

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144.02/139. ***Optogenetic inhibition of the infralimbic cortex following unreinforced lever presses increases cocaine seeking in rats***

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The infralimbic cortex (IL), region of the medial prefrontal cortex, is a component of the neural circuitry that mediates extinction learning and the active suppression of cocaine-seeking behavior. IL inactivation and activation immediately after extinction training impairs and enhances, respectively, the retention of extinction learning for cocaine seeking. However, the precise temporal relationship between IL activity, lever pressing, and extinction learning is unclear. Therefore, we examined whether selective IL inhibition immediately following each unreinforced lever press during extinction affected ongoing and subsequent cocaine-seeking behavior. The light-sensitive outward proton pump eArchT3.0 was selectively expressed in glutamatergic pyramidal neurons by injecting the adeno-associated virus encoding for eArchT3.0 under the CaMKII $\alpha$  promoter bilaterally into the IL of male Sprague-Dawley rats. Rats underwent a minimum of 12 days of cocaine self-administration, during which each active (right)

lever press resulted in an infusion of cocaine and the presentation of a light and tone cue. After each right lever press, the lever was retracted for 20 s. Rats then underwent 5 days of shortened (30 min) extinction sessions, during which active lever presses did not produce cocaine infusions or cues. During these shortened extinction sessions, the IL was optically inhibited for 20 s following each unreinforced active lever press. This was followed by 5 days of full-length (2 hr) extinction sessions that served as retention tests for the extinction learning. Optical inhibition increased active lever pressing during the 5 sessions in which the inhibition occurred but had no effect on lever pressing during the 7 full-length extinction sessions. In a control experiment, similar 20-s periods of optical inhibition were provided, but in a manner not contingent upon lever pressing. In this case, IL inhibition did not increase lever pressing during the session itself or on the subsequent extinction sessions. Following extinction, rats underwent cue-induced reinstatement tests, in which active lever presses resulted in the delivery of a light and tone cue but no optogenetic manipulations were given. Rats that had received IL inhibition during extinction showed potentiated cue-induced cocaine seeking, whereas rats that had received non-contingent IL inhibition did not show any change in cue-induced reinstatement. These results suggest that IL activity immediately following an unreinforced lever press contributes to the suppression of ongoing cocaine-seeking behavior and is important for the suppression of subsequent cue-induced reinstatement.

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144.03/140. ***Glucagon-like peptide-1 receptor activation in the ventral tegmental area or the nucleus accumbens attenuates cocaine seeking in rats***

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Glucagon-like peptide-1 (GLP-1) receptor signaling in the CNS is pharmacologically and physiologically relevant for energy balance control. The GLP-1 receptor agonist exendin-4 decreases intake of palatable food when administered into the ventral tegmental area (VTA) and nucleus accumbens (NAc) core. Since the VTA and the NAc mediate the reinforcing effects of food and drugs of abuse, we hypothesized that GLP-1 receptor activation in these two nuclei would attenuate cocaine reinstatement, an animal model of relapse in human addicts. Initially, rats were allowed to self-administer cocaine (0.25 mg/infusion i.v.) for 21 days on a fixed-ratio 5 (FR5) schedule of reinforcement. Cocaine self-administration was then extinguished by replacing cocaine with saline. Once cocaine taking was extinguished, rats received an acute priming injection of cocaine (10 mg/kg, i.p.) to reinstate cocaine-seeking behavior. During subsequent reinstatement test sessions, rats were pretreated with intra-cranial infusions of the GLP-1 receptor agonist exendin-4 (0, 0.005 and 0.05 µg) prior to a priming injection of cocaine. Here, we show that administration of exendin-4 directly into the VTA, NAc core or NAc shell dose-dependently attenuated cocaine priming-induced reinstatement of drug-seeking behavior. To determine if the suppressive effects of exendin-4 in the VTA and NAc on cocaine seeking were due to drug-induced motor impairments, we also examined the effects of intra-cranial exendin-4 infusions on the reinstatement of sucrose seeking. Administration of exendin-4 directly into the VTA, NAc core or NAc shell had no effect on sucrose reinstatement. Taken together, these results indicate that increased

activation of VTA and NAc GLP-1 receptors is sufficient to reduce cocaine seeking and that these effects are not due to general motor suppressant effects of drug treatment. Thus, these findings support re-purposing GLP-1 receptor agonists, which are FDA-approved for treating diabetes type II and obesity, for treating cocaine addiction.

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144.04/141. ***Hippocampal inputs to lateral septum drive context-induced, but not cue-induced cocaine seeking***

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Stimuli associated with drug experiences can trigger relapse in drug addicts. Drug-associated contexts and discrete drug cues initiate relapse by activating distinct brain regions. However, neural circuits distinctly involved in these different relapse modalities have not been fully characterized. Using a modified self-administration model, all rats self-administered cocaine with light/tone cues in one context, extinguished this behavior in an alternative context (without light/tone cues), and reinstated in either the training context without light/tone cues (context reinstatement, ABA) or in the extinction context with light/tone cues (cued reinstatement, ABB) to dissociate context vs. cued reinstatement of cocaine seeking. We then used Fos expression as a marker of neural activation in brain regions, a retrograde tracer combined with Fos to determine activated circuits, and pharmacologic and chemogenetic approaches to examine the role of hippocampal inputs to lateral septum (LS) in these reinstatement modalities. Based on the involvement of the hippocampus in contextual processing, its dense projections to LS, and previous results from our lab showing a functional CA3-LS-VTA circuit, we hypothesized that this circuit is important specifically for context-induced cocaine seeking, but not seeking driven by discrete cues. Results revealed that both dorsal hippocampus (CA1, CA3, and dentate gyrus) and LS (caudal and rostral LS) expressed a greater number of Fos cells during context compared to cued reinstatement. Furthermore, a greater percentage of CA3 neurons that project to LS express Fos during context compared to cued reinstatement or extinction, indicating this circuit is specifically activated during re-exposure to drug-associated contexts. Interestingly, pharmacological inhibition of LS attenuated both context and cued reinstatement. We then used DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) to specifically inhibit hippocampal (CA3) terminals in LS. Inhibitory (hM4Di) DREADDs virally transduced into CA3 were transported to terminals in LS and activated by local microinjections of the ligand clozapine-N-oxide (CNO). Inhibition of hM4Di-expressing CA3 terminals in LS attenuated context, but not cued reinstatement. Together these findings highlight the importance of LS in cocaine-seeking behavior, and that hippocampal inputs to LS drive context-induced reinstatement, whereas other inputs to LS likely drive cue-induced reinstatement. Elucidating the circuitry involved in different relapse modalities will identify therapeutic targets for specific relapse triggers in recovering drug addicts.

144.05/142 ***Drug seeking during initial abstinence is driven by hippocampal  $\beta$ -adrenergic and serotonergic receptors in a sex-dependent manner***

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Studies indicate that female rats exhibit greater drug seeking than male rats during initial drug abstinence. Moreover, females are more sensitive to the effect of stress to drive drug seeking than males. Locus coeruleus norepinephrine (LC-NE), dorsal raphe serotonin (DR-5HT) and corticotropin releasing factor (CRF) neurons are involved in stress responses, including the ability of stress to drive drug relapse. Notably, LC-NE neurons are more sensitive to CRF in females compared to males, and conversely, DR-5HT neurons are more sensitive to CRF in males compared to females. Dorsal hippocampus (DH) is a prominent focal point in the stress response that receives strong inputs from both LC-NE and DR-5HT neurons. DH has number of structural and biochemical sex differences that modulate stress responsivity, including substantial differences in CRF receptor binding affinity, de novo serotonin synthesis, cholinergic enzyme activity as well as adrenergic, corticosterone, and GABA receptor expression. Notably, DH is required for context-dependent reinstatement of drug seeking, and drug relapse often occurs when addicts are re-exposed to drug-associated contexts. Thus, we hypothesized that  $\beta$ -adrenergic and serotonergic neurotransmission in DH is involved differentially in male and female rats in drug seeking during extinction day (ED1), i.e., the initiation of abstinence when animals are re-exposed to the drug-associated context. Drug-seeking during this initial abstinence test was decreased by S-propranolol ( $\beta$ -adrenergic and 5-HT<sub>1A/1B</sub> receptor antagonist), R-propranolol (5-HT<sub>1A/1B</sub> receptor antagonist), and racemic (R/S mixture) propranolol in both male and female rats (10mg/kg, IP). We observed that hippocampal, locus coeruleus, and dorsal raphe Fos expression was increased on ED1 in both male and female rats, and that hippocampal Fos was decreased by systemic S-propranolol. Utilizing intrahippocampal infusions of S-propranolol, a Betaxolol/ICI-118,551 cocktail (selective  $\beta$ -adrenoceptor antagonists,  $\beta$ -AR), or a WAY-100635/GR-127935 cocktail (5-HT<sub>1a</sub> & 1b receptor antagonists), we investigated the role of hippocampal 5-HT and  $\beta$ -adrenergic neurotransmission in ED1 drug-seeking behavior. ED1 responding was reduced by 5-HT but not  $\beta$ -AR antagonists in males, and reduced by both 5-HT and  $\beta$ -AR antagonists in females. Thus, drug seeking during initial abstinence requires hippocampal 5-HT and  $\beta$ -AR neurotransmission in females, but only 5-HT neurotransmission in males. Additional studies are underway to test if manipulations of hippocampal serotonergic or adrenergic systems during ED1 influences later relapse to cocaine seeking.

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144.06/143. ***Attenuation of orexin/hypocretin signaling decreases cocaine seeking and increases cocaine taking in rats with a history of self-administering cocaine and ethanol***

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Abuse of psychostimulants such as cocaine has a high comorbidity with heavy use of alcohol with 85-88% of cocaine-dependent individuals engaging in co-use of alcohol. Such co-use is problematic given findings that the alcohol-cocaine combination is the most common substance use pattern reported in emergency rooms. The prevalent co-abuse of alcohol and cocaine leads us to hypothesize that alcohol abuse aggravates cocaine abuse, and facilitates the transition to cocaine addiction. Male, Sprague-Dawley rats were initially trained to drink ethanol in their home-cage using the intermittent access (IA) paradigm in which liquid ethanol (20%) was available every other day. Following two weeks of IA, subjects were trained to self-administer cocaine on within-session threshold behavioral economics (BE) procedure. A median split was performed on voluntary drinking behavior to sort animals into high drinkers (HD) and low drinkers (LD). Results showed that HD rats had significantly higher baseline demand elasticity for cocaine, indicating lower motivation to self-administer cocaine relative to the LD rats. Over time this difference between groups decreased, such that initial LD rats maintained the same demand elasticity for cocaine, whereas HD rats decreased their cocaine demand elasticity (increased motivation) over the course of the experiment. These results imply that HD rats are initially resistant to cocaine, but with repeated exposure to both cocaine and ethanol this demand for cocaine increases. We hypothesize that this may reflect trait differences in anxiety that are mitigated by alcohol consumption over time. To assess the roles of orexin signaling in free consumption and motivation for cocaine in cocaine-alcohol exposed subjects, rats from the above experiment were given an i.p. injection of the orexin 1 receptor antagonist SB334867 (30 mg/kg) 30 min prior to a cocaine BE self-administration test session. Results showed a significant increase in cocaine demand elasticity indicating a significant decrease in motivation for cocaine. Interestingly, a significant increase was also found in cocaine free consumption, indicating an increase in the desired level of cocaine in brain, or hedonic set point. These results indicate that attenuation of orexin signaling decreases motivation for cocaine (as observed previously in cocaine-only subjects) but increases cocaine free (low effort) consumption. Thus, alcohol plus cocaine exposure elicits adaptations in the orexin system not seen in subjects exposed to cocaine alone. Possible clinical implications of these adaptations deserve further study. Supported by PHS grants R01-DA006214 and F32-DA036995

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144.07/144. ***Long access self-administration increases cocaine demand: Dependence on orexin 1 receptor signaling***

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Orexin/hypocretin plays a key role in stimulus-driven drug-seeking. Blockade of the orexin 1 receptor (Ox1R) impairs cue-, context-, and stress-induced reinstatement. Using a novel behavioral economics procedure, our lab recently demonstrated that the selective Ox1R antagonist SB-334,867 decreases demand for cocaine, but only in high-demand animals trained with drug-paired cues (Bentzley & Aston-Jones, 2015). These results highlight the contribution of orexin to trait-based motivation for cocaine. Yet, cocaine dependence often results from state factors, rather than innate individual variations. The

current experiment uses this rationale to test if Ox1R antagonism can also attenuate motivation for cocaine in rats exhibiting escalated intake following long access self-administration. Male Sprague-Dawley rats were implanted with jugular catheters for i.v. cocaine self-administration. After initial FR1 self-administration training, subjects were trained until stable on the behavioral economics demand procedure (Bentzley et al., 2013). Rats were then trained for 14 days of long access (LgA; 6 Hrs) or short access (ShA; 1 Hr) FR1 self-administration. Following LgA or ShA self-administration, rats were given counterbalanced systemic injections of vehicle or two doses of the Ox1R antagonist SB-334,867 (SB; 10 and 3 mg/kg). Animals then went through extinction training followed by reinstatement tests with SB. As has been previously observed, LgA self-administration caused escalation of cocaine intake, particularly in the first hour. LgA also decreased demand elasticity and increased free consumption of cocaine (alpha and Q0 parameters), reflecting increased motivation and desired brain level for cocaine, respectively. SB altered these cocaine demand parameters in a dose-dependent fashion, towards pre-LgA values. These results demonstrate that the Ox1R is necessary for elevated cocaine demand following long access escalation. Whereas previous data from our lab show that Ox1R antagonism attenuates cocaine demand in animals with innate “trait-based” demand, the findings of the current experiments indicate that Ox1R blockade also decreases “state-based” demand following prolonged cocaine exposure. These findings have translational implications for cocaine addiction, wherein extended use triggered by environmental factors leads to a state of dependence that is difficult to treat. As LgA cocaine self-administration is anxiogenic and Ox1R blockade is known to be anxiolytic, we hypothesize that Ox1R blockade decreased cocaine demand by decreasing the anxiety involved in escalation of intake.

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144.08/145. ***Effects of central and peripheral oxytocin on reinstated cocaine seeking in male and female rats***

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Background: Oxytocin has gained increasing attention as a possible treatment for multiple neuropsychiatric disorders, including addiction. Oxytocin impacts natural and drug reward due to extensive innervation of central reward pathways. Sex differences clearly exist in psychostimulant addiction patterns. However, the underlying neurobiology and potential addiction therapies have typically only been studied in males. The oxytocin system is sexually dimorphic with a greater number of oxytocin receptors expressed throughout the addiction circuit in males relative to females. Here, we determined that oxytocin decreased reinstated cocaine seeking in males and females following systemic oxytocin treatment. We are currently investigating effects of intracerebroventricular (icv) administration of oxytocin with this paradigm. Methods: Male and female rats underwent 2 weeks of cocaine self-administration followed by extinction and reinstatement tests after systemic (1 mg/kg, i.p.) oxytocin or

vehicle treatment in the presence of conditioned cues. Following testing, rats were perfused and the brains were processed for c-Fos staining and c-Fos/oxytocin double-labeling in the paraventricular nucleus of the hypothalamus (PVN). A separate group of rats received oxytocin treatment during extinction and were tested with vehicle or oxytocin in response to cocaine-conditioned cues. Results: An acute oxytocin injection (i.p.) reduced reinstated cocaine seeking in both males and females. Likewise, repeated oxytocin during extinction also reduced responding on a cue test in males and females. However, oxytocin during extinction had no lasting impact on a cued reinstatement test. Currently, we are double labeling c-Fos and oxytocin cell bodies in the PVN to determine whether oxytocin reduced reinstated cocaine seeking via similar mechanisms. To date, we found males have a higher number of oxytocin and Fos-positive neurons in the PVN relative to females. Additionally, we are quantifying c-Fos expression in terminal areas. Discussion: Oxytocin impacted reinstated cocaine seeking similarly in both sexes. This similarity is in contrast to the well-known sex differences in the role of oxytocin on peripheral organ sites of actions and the sexually dimorphic distribution of central oxytocin receptor sites in females, relative to males. This study will determine if similar behavioral outputs may be mediated by a different underlying neurobiology.

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144.09/146. ***Treatment with the M1-selective muscarinic antagonist trihexyphenidyl attenuates cocaine-reinforced behavior***

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Medications that modify cholinergic tone can have pronounced effects on behaviors reinforced by natural and drug reinforcers. Systemic treatment with M1-preferential muscarinic agonists decreases self-administration across a broad range of cocaine doses. In contrast, M1-selective antagonists can enhance the discriminative-stimulus effects of cocaine. When administered with cocaine, M1 antagonists can potentiate increases in dopamine in the nucleus accumbens shell but not the core or prefrontal cortex. The present study examined effects of the M1-preferential muscarinic antagonist trihexyphenidyl (TXP) on cocaine- and food- reinforced behavior. METHODS: Rats were trained to respond for either cocaine or liquid food under a fixed-ratio-5 (FR-5) schedule during two-hour multiple-component sessions. Across components, either cocaine dose (0.1, 0.2, and 0.4 mg/kg per injection) or amount of 20% liquid food (30, 60, or 12  $\mu$ l) was varied. Pretreatment with TXP was administered intraperitoneally at low, intermediate, or high doses (1.0, 3.2, or 10 mg/kg-injection). RESULTS: TXP decreased cocaine-reinforced responding by 10 to 30% relative to baseline, with similar actions at different doses of TXP, as well as different cocaine doses. Responding for 30  $\mu$ l of liquid food was attenuated by high-dose TXP, but was otherwise unaffected. TXP increased spontaneous sniffing, rearing, and digging; without modifying inactive lever responding under any of the conditions tested. CONCLUSION: TXP can decrease drug self-administration across a broad, 10-fold range of cocaine doses. Effects on spontaneous behavior are less pronounced than for cholinergic agonists, and include

increases in some normally observed scored behaviors. These behavioral effects may be mediated by increases in dopamine in the nucleus accumbens shell.

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144.10/147. ***Pathological persistence of the brain response to “unseen” 33 msec cocaine cues as a marker of relapse vulnerability***

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**Aims:** Our laboratory has shown that even “unseen” (subliminal) 33 msec drug cues can trigger the brain’s subcortical motivational circuitry. Does this early brain response to drug cues constitute a relapse vulnerability? In a new cohort of treatment-seeking cocaine patients, we are examining whether the “pathological persistence” of the brain response to cocaine cues is linked to poor clinical outcome. **Methods:** In a “fast” event-related BOLD fMRI paradigm, cocaine inpatients were exposed to cocaine-related and to comparison (sexual, aversive and neutral) cues of 33 msec duration. Each cue (48 presentations of each cue category) was “backward-masked” by a 467 msec neutral stimulus to prevent conscious recognition. Pre-planned contrasts to characterize “persistence” (comparing the brain response during the first half vs. the second half of the task, for each cue category) were calculated within SPM 8 for two outcome subgroups “GOOD” (<30% cocaine-positive urines, n=9) vs. “POOR” (>90% cocaine-positive urines, n=15). **Results:** As hypothesized, cocaine patients with “POOR” clinical outcome evidenced a greater (e.g., drug<sub>2</sub> > drug<sub>1</sub>; p<0.05) response to 33 msec cues in the second half of the task, for (cocaine, aversive and neutral) of the cue categories [[unable to display character: &#8211;]] among a priori limbic regions, this “persistence” was reflected in amygdala/v. pallidum, and in temporal pole. “GOOD” outcome patients lacked this pattern. **Conclusions:** The current data highlight the relapse relevance of the early brain response to “unseen” cues”: patients with POOR outcome had cue-triggered brain responses that tended to persist -- despite the multiple, unreinforced presentation of the cues. “Pathological persistence” may be a sensitive predictor of relapse, complementing conventional “magnitude” measures in BOLD fMRI. These findings underscore the potential utility of the “unseen” cue paradigm, both as a tool for screening anti-relapse medications and for identifying the “cue-vulnerable” patients who need these medications most.

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144.11/148. ***The group metabotropic glutamate receptors regulation of cocaine seeking is receptor-selective and intake-dependent: Role of anatomical substrates***

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major obstacle in the treatment of addiction has been the propensity to relapse, often mediated by drug-associated cues, even after prolonged period of abstinence from drug use. Repeated exposure to cocaine leads to enduring alterations in glutamatergic signaling in the brain reward circuitry that play an important role in long-lasting molecular, cellular and behavioral neuroadaptations. Therefore, glutamate signaling has been investigated as a target for the development of treatment for addiction. Recent studies have suggested that the group I metabotropic glutamate receptors (mGluR1/5) play important roles in drug reinforcement and seeking and, therefore, have been pursued as promising targets for drug development. Here, we examined the role of mGluR1/5 receptors in abstinence drug seeking using animal models of cocaine self-administration. Sprague-Dawley rats were trained to self-administer cocaine (FR1; 1.0 mg/kg/200 µl/inf) during either 2-hr (ShA) or 6-hr sessions (LgA) for 14 days. Subsequently, animals were left undisturbed in home cage for 3, 10, or 60 days. Following abstinence period, rats were tested under extinction condition for cocaine seeking after either saline or an mGluR1/5 receptor antagonist administration (MTEP or JNJ16259685). Following a short abstinence period (3 or 10 days), the blockade of mGluR5 receptor reduced drug seeking only in ShA subjects without affecting the LgA animals, while mGluR1 receptor blockade were equally effective in reducing drug seeking in both groups. However, after a long abstinence period (60 days), the blockade of either of receptors significantly reduced drug seeking in ShA and LgA rats. Furthermore, mGluR5 blockade was effective in reducing drug taking (cocaine self-administration) in dose dependent manner by ShA subjects but not by LgA animals. The results suggest that exposure to cocaine produced a transient intake dependent plasticity in mGluR5 signaling in the brain. The observed plasticity is specific to mGluR5 signaling since blockade of mGluR1 receptors reduced drug seeking similarly in both ShA and LgA animals. In order to identify the anatomical substrates contributing to the selective modulation of mGluR5 signaling, site-specific blockade of mGluR5 receptors in the nuclei of motive circuit will be performed. The selective, intake dependent, and transient plasticity in brain mGluR5 signaling mediated by exposure to cocaine suggest an important role for mGluR5 in cocaine mediated neuroadaptations and addiction behaviors. Understanding the mechanism of cocaine mediated effects may reveal new molecular targets for therapeutic development for the treatment of cocaine addiction.

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144.12/J1 ***Orexin-A/hypocretin-1 in the paraventricular nucleus of the thalamus fails to reinstate cocaine-seeking behavior in animals with a history of cocaine dependence following a prolonged period of abstinence***

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Growing evidence implicates a role for orexin/hypocretin (Orx/Hcrt) neurons originating in the lateral hypothalamus (LH) and projecting to the paraventricular nucleus of the thalamus (PVT) in drug addiction. We previously reported that intra-PVT administration of orexin-A/hypocretin-1 (Orx-A/Hcrt-1) after 2 weeks of abstinence from cocaine or sweetened condensed milk (SCM) self-administration reinstated (primed) cocaine-seeking behavior in animals with short cocaine access (ShA, 2 h/day) or long

cocaine access (LgA, 6 h/day, an animal model of cocaine dependence), as well as SCM seeking-behavior, but with different dose-response profiles. Specifically, in LgA rats a left-upward shift of the dose-response function compared with SCM group and an upward shift compared with the ShA group were observed. This suggests that a history of cocaine dependence leads to neuroadaptive changes at the level of the PVT, resulting in the “sensitization” of PVT-Orx/Hcrt transmission, reflected by increased sensitivity (i.e., a leftward shift) and exacerbated behavioral responses (i.e., an upward shift) to the effects of Orx-A/Hcrt-1. The present study’s aim was to investigate whether the intra-PVT priming effect of Orx-A/Hcrt-1 is preserved following weeks of abstinence in animals that had cocaine self-administration history. Male Wistar rats were trained to self-administer ShA cocaine, LgA cocaine, or SCM for total of 2 days. After completion of the training procedure, the animals were maintained in their home cage for 2 weeks and then subjected to extinction training for 2 weeks (2 h/day). The following day, the rats received intra-PVT microinjections of 0.5 µg Orx-A/Hcrt-1 (a dose that produced equivalent reinstatement at weeks of abstinence in all groups), or the respective vehicle, and then placed into operant chambers under extinction conditions for 2 h. At 4 weeks of abstinence, intra-PVT Orx-A/Hcrt-1 produced reinstatement of both SCM and ShA that was identical to what was observed at weeks of abstinence. Surprisingly, Orx-A/Hcrt-1 did not trigger cocaine-seeking behavior in LgA rats. The data suggest that following cocaine dependence (i.e., LgA), functional changes in PVT Orx/Hcrt transmission occurred, reflected by a change in the pharmacological profile of Orx-A/Hcrt-1 at weeks (“sensitization” to the effects of Orx-A/Hcrt-1) and weeks (lack of Orx-A/Hcrt-1’s priming effects) of cocaine abstinence. One tentative explanation is that the expression and/or functionality of Orx/Hcrt receptors fluctuates during cocaine withdrawal following dependence, as reflected by decreased sensitivity to Orx-A/Hcrt-1’s priming effects.

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144.13/J2. ***Orexin-A/hypocretin-1 in the paraventricular nucleus of the thalamus induces Fos expression in the medial prefrontal cortex in animals with a history of cocaine dependence***

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Orexin/hypocretin (Orx/Hcrt) projections from the lateral hypothalamus (LH) to the paraventricular nucleus of the thalamus (PVT) are implicated in drug addiction. We previously reported that administration of orexin-A/hypocretin-1 (Orx-A/Hcrt-1) in the PVT reinstates extinguished cocaine-seeking behavior in animals with short access (ShA, 2 h/day) or long access (LgA, 6 h/day, a model of cocaine dependence) to cocaine and sweetened condensed milk (SCM) seeking, but with different dose-response profiles. Specifically, in the LgA group a left-upward shift of the dose-response function compared with the SCM group and an upward shift compared with the ShA group were observed. This suggests that a history of cocaine dependence leads to neuroadaptive changes at the level of the PVT, resulting in “sensitization” of PVT-Orx/Hcrt transmission, reflected by increased sensitivity (i.e., a leftward shift) and exacerbated behavioral responses (i.e., an upward shift) to the effects of Orx-A/Hcrt-1. The present study sought to investigate whether the neural activation pattern following intra-PVT

Orx-A/Hcrt-1 administration in animals that self-administered cocaine (ShA, LgA) or SCM is different and could partially explain the different Orx/Hcrt dose-response functions. Male Wistar rats were trained to self-administer short-access cocaine (ShA), long-access cocaine (LgA), or SCM for a total of 21 days. After completion of the training procedure, the animals underwent extinction training for 2 weeks in 2 h daily extinction sessions. Rats received intra-PVT microinjections of Orx-A/Hcrt-1 (0.5 µg) or the respective vehicle (saline) and then placed into operant chambers under extinction conditions for 2 h. At the end of the behavioral tests, the brains were prepared for Fos immunohistochemistry and analyzed for Fos expression in the medial prefrontal cortex (mPFC), nucleus accumbens (NAC) core, and shell (i.e., brain regions receiving inputs from the PVT and well known to regulate cocaine-seeking behavior). Intra-PVT administration of Orx-A/Hcrt-1 induced strong activation of the mPFC (i.e., increased Fos-expressing neurons) only in animals that had a history of cocaine dependence. In contrast, no significant activation was found in the NAC shell and core, with no differences between groups. These data suggest that the LH→PVT→mPFC pathway, through Orx/Hcrt transmission at the PVT interface, is a neuronal circuit that drives cocaine-seeking behavior in cocaine-dependent animals.

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144.14/J3 ***Experience-dependent changes in calcium-permeable AMPA receptors in the mPFC following cocaine conditioned place preference versus cocaine self-administration***

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Relapse to cocaine seeking involves corticostriatal neurotransmission. Plasticity of glutamatergic synapses is a fundamental mechanism through which experience changes neural function to impact behavior. Here, we tested whether experience-dependent changes in levels of cortical Ca<sup>2+</sup>-permeable AMPA receptors (Cp-ARs) depend on re-exposure to cocaine-associated context. AMPA receptor rectification index (RI) was calculated as  $[RI = (eEPSC \text{ amplitude at } -70mV)/(eEPSC \text{ amplitude at } +40mV)]$  from either prelimbic (PL-mPFC) or infralimbic (IL-mPFC) medial prefrontal cortex deep layer pyramidal neurons. We found that the RI was significantly decreased in PL-mPFC neurons and trended towards an increase in IL-mPFC neurons of rats that underwent cocaine self-administration, extinction and cue-induced reinstatement. However, this methodology does not allow for a distinction between discrete cues versus contextual mediated effects. To address this distinction we used a cocaine conditioned place preference (CPP) model to establish a contextual associative memory and address whether Cp-ARs are modulated by re-exposure to cocaine-associated environment. In the first experiment, rats underwent conditioned place preference. On day 1, rats received a 10 minutes pre-conditioning test where they had free access to the entire apparatus. During the 7 days of conditioning, rats received daily single injections of either cocaine (20mg/kg i.p.) paired with a distinct compartment or saline paired with another compartment for 2 minutes. On the test day, animals were given free access to both compartments in a drug-free state and their preference was assessed for 10 minutes. After the first CPP test, animals were split into two groups: 8 days or 30 days of abstinence. These groups are further subdivided into rats that are either tested or not tested and killed 15 minutes later for whole-cell

voltage clamp experiment. Our results show a increase in RI after 8 days of abstinence, 8 days of abstinence followed by CPP test and 3 days of abstinence compared to saline controls. Interestingly, testing the rats for CPP after 30 days of abstinence lead to a decrease in RI. In the second experiment, rats underwent 10 days of cocaine self-administration (2mg/50µL). Following weeks of abstinence, rats were killed without testing and we found no difference between saline controls RI values in PL and IL-mPFC neurons. This was not surprising given that the rats did not re-experience the drug associated context or discrete cues. Future work will parse apart the importance of the cue v context association as well as the influence of abstinence vs extinction.

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144.15/J4. ***Involvement of the dorsal hippocampus and HDAC3 in cocaine drug-seeking***

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Substance use disorder is chronic, often relapsing disease that leads to a loss of behavioral inhibition and compulsive drug-seeking. Cues that are paired with acquisition of drug-seeking are thought to influence subsequent extinction (animal model of exposure-based therapy) and relapse-like behavior, both in humans and animals. Our previous work has implicated the dorsal hippocampus for developing and retrieving memories in drug-seeking context. By inactivating the dorsal hippocampus, we found that extinction was impaired in a contextual learning paradigm (cocaine-induced conditioned place preference). In addition, the epigenetic enzyme histone deacetylase 3 (HDAC3), has been shown to be a negative regulator of cocaine-associated learning and memory. Here, we further investigate this hippocampal-based extinction model and determine whether inhibition of HDAC3 can enhance extinction after cocaine self-administration. Despite the fact that extended extinction does not eliminate contextual renewal or cue-induced reinstatement, we find that a systemic injection of a synthetic HDAC3 inhibitor creates persistent extinction and weakens renewal and cue-induced reinstatement. In addition, we test whether inhibition of HDAC3 in the dorsal hippocampus alone is sufficient to impair extinction. In contrast to our systemic manipulation, it appears that dorsal hippocampus-specific HDAC3 inhibition does not alter drug-seeking behavior. Results suggest that regions outside of the dorsal hippocampus likely contribute to HDAC3-mediated enhancements in extinction after cocaine self-administration.

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144.16/J5. ***Inhibitory influence of basolateral amygdala cannabinoid CB1 receptors in instrumental cocaine memory reconsolidation***

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Exposure to cocaine-associated context can reinstate extinguished drug-seeking behavior and trigger the reactivation of contextual cocaine memories. Reactivated cocaine memories are labile and sensitive to manipulation until they are re-stabilized into long term memory stores through the process of memory reconsolidation. Our laboratory has shown that cannabinoid 1 receptors (CB1R) play a role in instrumental cocaine memory reconsolidation. In the present study, we evaluated the specific contribution of CB1R populations in the basolateral amygdala (BLA) to this phenomenon. We trained rats to lever press for cocaine infusions in a distinct environmental context followed by extinction training in a different context. In order to reactivate cocaine memories, the rats were re-exposed to the previously cocaine-paired context for 15 min. The selective CB1R antagonist, AM251 (300 ng/hemisphere), or vehicle was microinfused bilaterally into the BLA either immediately or 6 h following memory reactivation (i.e., outside of the time window of memory reconsolidation). Seventy-two h later, cocaine-seeking behavior (i.e., non-reinforced active lever presses) was assessed in the previously cocaine-paired context. Remarkably, intra-BLA AM251 administration immediately, but not 6 h, following cocaine memory reactivation facilitated subsequent cocaine-seeking behavior. This suggests that the stimulation of CB1Rs in the BLA inhibits instrumental cocaine memory reconsolidation. Thus, BLA CB1Rs may be novel therapeutic targets for disrupting the salience or intrusiveness of maladaptive drug memories and for preventing relapse.

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144.17/J6. ***Time-dependent, opposite effects of glucocorticoid receptor antagonism in the basolateral amygdala on drug context-induced cocaine-seeking behavior***

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Drug context-induced relapse to cocaine-seeking is dependent on the integrity of context-cocaine associative memories and the recruitment of these memories and other processes that trigger goal-directed behavior. Here, we investigated the role of basolateral amygdala (BLA) glucocorticoid receptors (GR) in A) contextual cocaine memory reconsolidation, a process responsible for long-term memory maintenance, and B) the drug context-induced reinstatement of cocaine-seeking behavior. Rats were trained to lever press for cocaine infusions in a distinct context followed by extinction training in a different context. In Exp. 1, rats received bilateral intra-BLA microinfusions of the GR antagonist, RU038486 (3, 10 ng/hemisphere), or vehicle following exposure to the previously cocaine-paired context, a procedure that elicits cocaine memory reactivation and reconsolidation. Controls were exposed to an unpaired context (no reactivation control). Non-reinforced lever presses were assessed 7 h later in the cocaine-paired context. In Exp. 2, rats received the same pharmacological manipulations immediately prior to testing. Intra-BLA RU038486 administration dose-dependently increased cocaine-seeking behavior 72 h later. This effect did not depend on memory reactivation; therefore, it did not indicate enhancement in memory reconsolidation. This RU038486-induced increase in cocaine-seeking behavior was associated with a paradoxical decrease in BLA glutamate GluN2a and

GluN2b subunit activation, which in turn positively correlated with GR levels and ERK1/2 activation, respectively. Intra-BLA RU038486 administration at test dose-dependently attenuated drug context-induced cocaine-seeking behavior. Furthermore, intra-BLA RU038486 failed to alter locomotor activity immediately or 72 h after administration. Together these findings suggest that BLA GR stimulation is necessary for drug context-induced motivated behavior. However, compensatory changes precipitated by BLA GR antagonism can result in a protracted increase in cue reactivity.

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144.18/J7. ***Optogenetic inhibition of the dorsal hippocampus: Effect on reconsolidation of cocaine-associated contextual memories and subsequent cocaine-seeking behavior***

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Cocaine addiction is a chronic relapsing disorder. Relapse can be triggered by drug-associated environmental stimuli, suggesting that maladaptive cocaine-associated memories contribute to the addiction cycle. The maintenance of these memories over time depends on their reconsolidation into long-term memory stores following reactivation-induced de-stabilization. We have shown that tetrodotoxin (TTX)-induced inactivation of, but not protein synthesis inhibition in, the dorsal hippocampus (DH) impairs cocaine memory reconsolidation and subsequent cocaine-seeking behavior. Accordingly, the DH may maintain labile memories prior to their re-stabilization at another site, such as the amygdala. Therefore, the DH may be more critical during the initial stages of the 4-hour memory reconsolidation window. We have begun to examine the temporal dynamics of DH recruitment for cocaine memory reconsolidation and hypothesized that optogenetic inhibition of the DH during the first hour following memory reactivation would impair memory reconsolidation. Sprague-Dawley rats received bilateral infusions of AAV5-hSyn-eArch3.0-YFP plus optic fibers into the DH. Rats were trained to lever press for un-signaled cocaine infusions in a distinct context and underwent extinction training in a different context. Following extinction training, rats were re-exposed to the previously cocaine-paired context for 15 minutes in order to de-stabilize cocaine-associated memories and trigger memory reconsolidation. Rats were then placed in a third context, where they received bilateral laser stimulation (532 nm, 1 second on/off) or no stimulation for 1 hour. After 2 additional extinction training sessions, cocaine-seeking behavior (i.e., non-reinforced lever presses) was assessed in the cocaine-paired context. Optogenetic inhibition of the DH during the first hour following re-exposure to the cocaine-paired context was sufficient to impair subsequent cocaine-seeking behavior. This suggests that the DH plays a critical role in the early stages of cocaine memory reconsolidation.

144.19/J8. ***Retrograde contextual learning induced by cocaine in a conditioned place preference paradigm***

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The purpose of these studies was to evaluate the magnitude of appetitive trace conditioning of context that could be induced by cocaine when presented at different interstimulus intervals (ISI) following a contextual S+. Swiss-Webster mice were tested using a standard conditioned place preference (CPP) procedure in which simultaneous pairing of 15 mg/kg cocaine with a distinctive, non-preferred floor type (S+) (composed of stainless steel rods or perforated holes) yielded a CPP. Mice were assigned to either cocaine control condition (receiving simultaneous pairing with the S+ floor), or to trace pairing, with cocaine administered in the home cage after an ISI of 0.25, 4, or 8 h following exposure to the S+ floor. Additional mice were assigned to a saline control (null) condition in which no cocaine was administered. On two consecutive days, all mice were injected with saline before morning session on the S- floor. During an afternoon session on the S+ floor, the mice received either saline (saline control and trace pairing groups) or 1 mg/kg cocaine (cocaine control group). Following each afternoon session, mice were returned to their home cages in the vivarium where they received third injection of either saline (saline control, cocaine control) or cocaine (0.25-h ISI, 4-h ISI, 8-h ISI). Preference test was conducted on the next day in which all mice were injected with saline and placed in the apparatus with split floors consisting of rods and holes, and the time spent on each floor was recorded. A positive shift in preference for the S+ floor was considered to reflect appetitive conditioning to the context. Three of the groups of mice exposed to cocaine (cocaine control, 0.25-h ISI, 4-h ISI) expressed a strong preference for the initially non-preferred floor. However, the shift in preference for S+ was absent in the 8-h ISI group. These studies suggest that retrograde appetitive conditioning of cocaine may occur to a salient context that was present up to 4 hours prior to cocaine exposure. The unusually long time-frame of this retrograde interaction is similar to that observed in studies of conditioned taste aversion, and this property may account for the important role of context in psychostimulant addiction and relapse.

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258.05/Y16. ***Basolateral amygdala mu opioid receptor activation and connections to the orbitofrontal cortex mediate outcome-specific Pavlovian-instrumental transfer***

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Environmental reward-predictive stimuli provide a major influence over reward-seeking behavior. The basolateral amygdala (BLA) is involved in this, and is particularly critical for situations in which such cues provide reward-specific information that allows them to selectively invigorate and bias selective actions. But how the BLA functions within a larger circuit to carry out this complex function is largely unknown. The BLA shares dense and reciprocal excitatory connections with several cortical areas, including the

orbitofrontal cortex (OFC), which is itself implicated in the ability of environmental cues to convey information about anticipated rewards. Here using the outcome-specific Pavlovian-instrumental transfer (PIT) task we evaluated the role of OFC-BLA circuitry in the selective invigorating and action selection biasing effects of reward-predictive cues over reward seeking. Pharmacological disconnection of these structures by contralateral transient inactivation impaired the ability of reward-predictive cues to, in a choice test, selectively invigorate the performance of actions that earned the same specific reward associated with the stimulus. Unilateral inactivation of either structure and ipsilateral OFC-BLA inactivation were without effect. Interestingly, OFC-BLA disconnection appeared to spare the action-selection biasing effect of the cues; rats were able to choose actions on the basis of the specific reward predicted by the cue, but the performance of this action was not invigorated above baseline response levels. To explore the specific mechanisms of this within the BLA we examined the role of BLA opioid receptors in PIT. While selective blockade of the delta opioid receptor was without effect, blockade of BLA mu opioid receptor activity abolished both the selective excitatory and response-biasing effect of reward-predictive cues over reward-seeking actions. These data suggest that connections between the OFC and BLA are vital for representing specific rewards, in this case provided by Pavlovian conditioned stimuli, and using this information to guide reward seeking, with mu opioid receptor activation in the BLA potentially working to modulate this excitatory circuit.

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258.06/Y17. ***Comparison of rapid dopamine signaling dynamics in the nucleus accumbens core and shell during a magnitude-based decision making task***

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To maximize resources, organisms must choose actions that result in the most valuable outcome available and maintain that information to guide future behaviors. Integral to this decision making process is the mesolimbic dopamine system, including the nucleus accumbens (NAc) and its dopaminergic input from the VTA. In the rat, the NAc is divided into two discrete subregions, the core and the shell, believed to process information about reward learning, reward value, and decision making related to goal-directed actions. However, the precise role of those subregions in processing information about magnitude-based decisions remains unclear. Here, dopamine (DA) release was first measured in the NAc shell using fast-scan cyclic voltammetry (FSCV) during a magnitude-based decision making task. Male Sprague-Dawley rats (n= 7) were trained to lever press following distinct visual cues that predicted the magnitude of future rewards. On Forced Choice Low Magnitude trials, a cue light predicted the opportunity to press a lever for a small reward (one 45mg sucrose pellet). On Forced Choice High Magnitude trials, another distinct cue light predicted the opportunity to press a different lever for a large reward (two 45 mg sucrose pellets). Lastly, on Free Choice trials, both cue lights and levers were presented and rats were able to choose between both magnitude options. All rats accurately discriminated between cue types on Forced Choice trials and developed preferences for the high magnitude option on Free Choice trials. Electrochemical recordings from electrodes in the NAc shell

show increases in rapid DA release following presentation of Forced Choice cues, with peak DA concentrations being significantly greater during the high forced cue. However, results on the Free Choice trials indicate that peak DA during the choice cue was the same regardless of whether the animal subsequently chose the large or small magnitude option. In an ongoing second study, rapid dopamine release is being monitored in the NAc core in another set of rats during the same task. Preliminary results (n= 2) suggest a similar trend in dopamine release dynamics in the NAc core to Forced and Free Choice cues. The current findings implicate the NAc shell in encoding comparative reward value during reward magnitude-based decision making, and ongoing studies will confirm if the NAc core plays a similar role in this process. Supported by NIH DA034021 to RMC.

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258.07/Y18. ***Examination of a history of cocaine self-administration on rapid dopamine signaling during delay discounting task***

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Cocaine use has been associated with heightened impulsivity in both humans and rodents. However, the precise neuronal mechanisms through which these two behaviors are related remains unclear. Several brain regions integral to impulsive control are altered by cocaine use, including the nucleus accumbens (NAc) and its dopaminergic input. Indeed, prior work from this lab has shown that rapid dopamine (DA) signaling in the NAc core tracks reward value during delay discounting, an impulsive decision making task. To examine the relationship between cocaine history, impulsive behavior and rapid DA release dynamics, we trained animals (n= 24) in a delay discounting task where they learned that distinct cues signaled the opportunity to choose between a small reward available immediately after a response versus a large reward that was available after either no delay (0 sec), a short delay (10 sec), or long delay (20 sec). Next, rats were trained to self-administer either intravenous cocaine (n=12) or water (n=12) during 2 hr daily sessions for two weeks. Animals were tested on the delay discounting task immediately after completion of the two weeks of self-administration and following an additional week abstinence period (rats put in home cage, n drug). In a subset of rats (cocaine: n = 4; water: n = 3), rapid DA release was measured in the NAc core using fast-scan cyclic voltammetry during delay discounting. All rats exhibited typical delay discounting behavior, shifting preference from the large reward to the small reward as the delay to the large reward increased. Contrary to other findings, however, a history of cocaine self-administration did not significantly alter delay discounting behavior compared to water controls when tested either immediately after completion of the two weeks of self-administration or after an additional week abstinence period. Nonetheless, consistent with prior work in our lab (Saddoris et al., Biol Psych, 2014), cues predictive of available choices evoked DA release that scaled with the rat's preferred choices and dynamically shifted as delay to reinforcement for the large reward increased. Interestingly, although a history of cocaine did not alter delay discounting behavior, cocaine-experienced rats released significantly less DA to the cues signaling available choices in the task than did water rats. These preliminary data suggest that while a history of cocaine self-administration did not

make rats more impulsive, it did dampen DA processing of cues that signaled the availability of small immediate versus large delayed rewards. Supported by: DA034021

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258.08/Y19. ***Nucleus accumbens subregions (core vs shell) differentially encode reward-associated cues following reinforcer devaluation***

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Nucleus accumbens (NAc) neurons encode features of stimulus learning and action selection associated with rewards. Additionally, the NAc is necessary for using information about expected outcome values to guide behavior as measured by reinforcer devaluation tasks. Further, evidence suggests that the distinct subregions of the NAc (core and shell) may play unique roles in guiding motivated behavior. Here, we recorded neural activity in the NAc core and shell during training (after cue-outcome associations were established) and performance of reinforcer devaluation task. Specifically, male Long-Evans rats ( $n=25$ ) were trained to press lever following an illuminated cue light that predicted a specific reinforcer (e.g., raspberry flavored pellet). Rats received an alternative reinforcer in their home cages following training (e.g., peanut butter food pellets). Once rats achieved 90% accuracy during training, they were probed in devaluation test under extinction conditions. Specifically, each rat was allowed ad libitum access to one of the two foods (selective satiation). On separate day, the other food was devalued (counterbalanced). Rats lever pressed significantly less when the same reinforcer received during training was devalued ( $66.9 \pm 8.6$ ) compared to devaluation of the alternative reinforcer (nondevalued,  $86.8 \pm 9.3$ ;  $p < .05$ ) showing successful outcome specific devaluation. We recorded NAc neural activity on the last day of training, as well as the two test days (devalued vs nondevalued). We found that in the NAc core (but not shell) there was a significant correlation between the percentage of neurons that encoded the cue on the last day of training and subsequent behavioral performance following outcome devaluation (i.e., the ability of rats to stop responding when the outcome had been devalued). Further, in the NAc shell (but not core), we observed a significant decrease in the percentage of NAc neurons that showed phasic responsiveness (i.e., cells that either increased or decreased firing) to the reward-associated cue when the same reinforcer received during training was devalued (5 out of 79, 6%) compared to the satiation of the alternative reinforcer (nondevalued, 2 out of 86, 24%). These data suggest that NAc core and shell neurons differentially encode information about reward-associated cues following outcome devaluation. Specifically, NAc core neural encoding during training predicts behavioral performance on subsequent test days. In contrast, the NAc shell dynamically encodes information about the cue with respect to the current value of the outcome.

258.14/Y25. ***Opposing roles for dopamine D1 and D2 receptor expressing accumbens medium spiny neurons in cocaine induced neuroplasticity and reinstated cocaine seeking***

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Medium spiny neurons (MSN) in the nucleus accumbens link internal motivational states and environmental information to motor action within the addiction circuit. Classically, dopamine D1-receptor expressing MSNs in this region are thought to drive motivated behavior through their projections to the ventral mesencephalon. In contrast D2-expressing MSN are canonically thought to inhibit and refine motivational drive through a circuit comprising the ventral pallidum (VP) and subthalamic nucleus. Recent data from our lab demonstrates, however, that both D1- and D2-expressing neurons project to the VP and that their projection from nucleus accumbens to ventral pallidum, but not to ventral mesencephalon is critical for reinstatement of cocaine seeking behavior (Stefanik et al., 2013). In addition, we recently demonstrated that after withdrawal from cocaine self-administration GABA-mediated LTD is lost in accumbens to ventral pallidum synapses due to an elevated enkephalin tone onto presynaptic  $\mu$ -opioid receptors (Kupchik et al., 2014). These observations demonstrate that the circuitry driving cocaine related behavior is more nuanced than previously thought. To assess whether cocaine self-administration diminishes LTD in D1- or D2-MSN projections to the VP, we expressed channelrhodopsin 2 in the nucleus accumbens of D1-Cre or D2-Cre driver mouse lines. Following ten days of cocaine self-administration and subsequent extinction acute brain slices were taken and LTD protocol was run while recording from VP neurons. In cocaine-extinguished mice,  $\mu$ -opioid driven LTD was abolished in the D2-to-VP projection while the D1-to-VP projection was unaffected, suggesting that cocaine selectively alters D2-driven plasticity in the VP. To test the hypothesis that altered D2-MSN function plays a role in the motivation to seek cocaine, we selectively expressed designer receptors exclusively activated by designer drugs (DREADDs, activated by clozapine-N-oxide) in D1-Cre or D2-Cre mice to modulate the activity of D1- or D2-MSN at the level of the nucleus accumbens. Inhibiting D2-MSN using the inhibitory Gi-coupled DREADD hM4D strongly potentiated cue induced reinstatement of cocaine seeking while inhibiting D1-MSN did not have a profound effect. Conversely, preliminary data suggests that activation of D1-MSN using the excitatory Gs-coupled rM3D DREADD drives reinstated behavior. Future studies will be aimed at the dissection of D1-to-VP and D2-to-VP contributions to cue induced cocaine seeking at the level of the ventral pallidum.

258.15/Y26. ***Junk food consumption induces abnormal food-seeking responses to environmental stimuli in rats***

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The pervasiveness of highly palatable, energy-dense foods in modern diets is considered a leading cause of obesity, which has become a significant public health concern. While the legitimacy of “food addiction” is currently under debate, overeating shares many characteristics with drug addiction, such as compulsive pursuit despite potentially dire health consequences. Cues associated with palatable foods (e.g., auditory cues such as commercial jingles, etc.) can trigger food-seeking, even when sated, which could lead to decreased inhibitory control over food intake. Here, we explored whether a junk food diet dysregulates rats’ ability to respond appropriately to food-paired cues in a Pavlovian-to-instrumental transfer test. Rats were first trained to lever press for a food reward. Subsequently they learned to associate free delivery of the reward with an auditory cue. Rats were then exposed to either normal chow (Control rats) or chow and a junk food diet for either 2 hrs (Binge rats) or 24 hrs (All-Day rats) per day, for up to 6 weeks. At test, rats were sated for 1 hour on chow, and presented with food-paired (CS+) and neutral (CS0) cues, and lever presses and food-cup entries recorded. Control rats increased lever pressing and food-cup entries at the onset of the CS+, but not the CS0, as expected. Binge rats increased lever pressing to both the CS+ and the CS0, though food-cup entries increased only during the CS+. All-Day rats showed markedly reduced lever pressing and food-cup entries to both cues. These results suggest that chronic junk food consumption induces atypical responding to environmental stimuli predictive of food rewards, and that different dietary access produces unique behavioral abnormalities in response to environmental stimuli. Specifically, Binge rats appear to generalize the excitatory properties of reward-paired cues to other, neutral cues, inappropriately triggering food-seeking, while All-Day rats appear insensitive to the motivational properties of the CS+. These data emphasize that junk food diets induce aberrant food-seeking in response to environmental cues, and this action may contribute to maladaptive feeding behavior leading to obesity.

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258.19/Y30. ***novel hypocretin receptor 1 antagonist, RTIOX-276, regulates cocaine self-administration and dopamine signaling in the nucleus accumbens core***

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Accumulating evidence indicates that the hypocretins / orexins (HCRT) influence cocaine reinforcement via actions on the mesolimbic dopamine (DA) system. We previously demonstrated that blockade of the

hypocretin receptor 1 with SB-334867 attenuates cocaine self-administration and reduces cocaine-induced enhancement of dopamine signaling in the nucleus accumbens core. The current study sought to assess the utility of a novel hypocretin receptor 1 antagonist, RTIOX-276, which has higher affinity and greater specificity for the hypocretin receptor than SB-334867. We examined the effects of RTIOX-276 on self-administration of cocaine under progressive ratio schedule of reinforcement. We also assessed whether RTIOX-276 decreases the effects of cocaine on dopamine signaling in the nucleus accumbens core using in vivo fast scan cyclic voltammetry. Results suggest that RTIOX-276 attenuates the motivation to self-administer cocaine and decreases cocaine-induced enhancement of dopamine signaling. Together with previous work using SB-334867, the current findings provide further evidence for hypocretin receptor 1 involvement in the regulation of reward and reinforcement processes, particularly as it relates to cocaine. Therefore, the hypocretin receptor 1 may be a viable target for development of pharmacotherapies for the treatment of cocaine addiction.

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258.20/Y31. ***new tool to exam molecular alterations in synapses of neuronal ensembles***

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Learned associations between drug effects and stimuli in the drug environment (context) play an important role in drug addiction and are thought to be encoded by sparsely distributed patterns of neurons called neuronal ensembles that are selected by the drug-related stimuli. We are now developing methods for assessing unique alterations within pre- and post-synaptic components of synapses onto these activated neuronal ensembles. To selectively label post-synaptic components on only activated post-synaptic neuronal ensembles, we injected AAV1 virus with the transgene CMV::DIO-PSD95\_Myc into nucleus accumbens of c-fos-tetop::iCre transgenic rats and injected the rats with 30 mg/kg cocaine four weeks later. The neural activity-dependent c-fos promoter in the rat transgene induces Cre recombinase protein expression that activates the DIO-PSD95-Myc viral gene in only strongly activated accumbens neurons. The fusion protein PSD95-myc is translocated to post-synaptic dendritic spines. Immunohistochemical labeling of Myc peptide indicated high levels of expression of PSD95\_Myc in post-synaptic dendrites of activated neurons one week after the cocaine injection. To selectively label vmPFC presynaptic terminals in accumbens, we injected AAV1 virus with the transgene EF1 $\alpha$ ::Synaptophysin\_Flag into vmPFC into wild-type rats. The fusion protein synaptophysin\_Flag is translocated to presynaptic terminals. Immunohistochemical labeling of Flag peptide indicated high levels of expression of synaptophysin in vmPFC terminals in accumbens four weeks after the virus injection. Expression of both fusion proteins was confirmed using Western blotting and flow cytometry. For flow cytometry, synaptoneurosomes containing pre- and post-synaptic components were obtained from nucleus accumbens of virus-injected rats and immunolabeled for PSD95-Myc and Synaptophysin-

Flag. Flow cytometry indicated expression of both fusion proteins in the nucleus accumbens as well as double-labeling of synaptoneuroosomes with PSD95-Myc and Synaptophysin-Flag. Identification of synapses on activated neurons will allow us to assess unique synaptic alterations on activated neuronal ensembles that mediate context-induced reinstatement of ethanol-seeking.

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**180.01/BB19. *Nucleus accumbens core muscarinic and nicotinic acetylcholine receptors differentially modulate phasic dopamine release and mediate cue-induced incentive motivation***

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Recent primarily in vitro data has demonstrated that within the striatum muscarinic and nicotinic acetylcholine receptors differentially modulate dopamine signaling. The working hypothesis is that striatal acetylcholine levels acting at nicotinic receptors on dopamine terminals act as a low-pass filter on phasic dopamine signaling, attenuating release when acetylcholine levels are high, and facilitating release when levels are low. Muscarinic receptors located on striatal cholinergic interneurons regulate acetylcholine release and therefore regulate this terminal modulation of dopamine release. Whether this occurs in awake, behaving animals and, vitally, whether such modulation has functional consequences related to motivated behaviors is currently unknown. Phasic dopamine is robustly released in the nucleus accumbens core (NAc) in response to reward-paired cues and such release tracks the incentive motivational impact of these cues- their ability to elicit excitation/arousal and to enhance non-selective range of reward-seeking actions. Therefore, here we used the Pavlovian-instrumental transfer (PIT) task to evaluate the hypothesis that activity at NAc acetylcholine receptors terminally modulates the phasic release of dopamine to mediate the ability of reward-paired cues to invigorate reward-seeking actions. First, we evaluated the role of NAc muscarinic and nicotinic acetylcholine receptors in PIT and found opposite contributions. Blockade of NAc nicotinic receptors (with intra-NAc mecamylamine) enhanced the invigorating influence of a reward-paired cue over reward-seeking actions, while blockade of muscarinic receptors (with intra-NAc scopolamine) selectively attenuated this effect. To determine if these effects were mediated through modulation of dopamine release, in a second experiment we monitored phasic NAc dopamine concentration changes with fast-scan cyclic voltammetry in rats behaving in the PIT task following unilateral infusion of either mecamylamine, scopolamine or ACSF vehicle directly into the recording zone. Early results suggest that local blockade of NAc nicotinic receptors accentuates and blockade of NAc muscarinic receptors attenuates cue-induced phasic dopamine release during PIT. These preliminary data support the hypotheses from in vitro data and provide potential functional role for acetylcholine modulation of dopamine release in mediating the excitatory influence of reward-paired cues over reward-seeking actions.

180.08/BB26. ***Individual variability in dopamine transporter regulation of neurotransmission and incentive motivation***

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Conditioned stimuli (CSs) have the capacity to powerfully motivate behavior by producing conditioned responses (CRs). There is considerable individual variation in the degree to which a CS exerts motivational control over behavior. For example, in an autoshaping animal model, a lever CS that predicts food reward (unconditioned stimulus, US) becomes attractive, wanted, and elicits an approach CR in a subset of outbred rats termed “sign-trackers” (ST), whereas “goal-tracker” (GT) rats exhibit a CR toward the location where the reward is delivered. Dopamine (DA) neurotransmission in the nucleus accumbens (NAc) core mediates the attribution of motivational meaning to the CS: it is required for lever-directed approach in ST rats, but not conditioned responding in GTs, even though all rats learn the stimulus-reward relationship equally well. Using in vivo fast-scan cyclic voltammetry (FSCV) recordings of DA release in the NAc core, we first replicated earlier studies (Flagel et. al. 2011) demonstrating CS-evoked DA responses during lever approach in STs. In contrast to STs, GTs displayed both CS- and US-evoked DA release during approach to the food-receptacle. A series of experiments were then conducted to better understand what mechanisms may account for differences in DA signaling in the NAc core. It was found that injection of AMPH directly into the NAc core selectively enhanced lever-directed approach in STs, amplifying the incentive value of the CS. This was hypothesized to result from increased DA transporter (DAT) binding of AMPH in STs, as STs showed elevated ventral striatal DAT surface expression compared to GTs. DAT regulation over DA was further investigated using ex vivo FSCV recordings of electrically-evoked DA release. STs showed more rapid DA uptake compared to GTs, supporting enhanced DAT function in STs. We propose that greater DAT surface expression in STs increases control over synaptic DA, enhancing the ability of phasic DA to induce plasticity that underlies the encoding of attraction to a reward paired cue (incentive motivation).

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180.11/BB29. ***Investigating contributions of dopamine D2 and D3 receptors to Pavlovian conditioned approach behaviors***

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Environmental cues that are repeatedly paired with reward can guide behavior in an adaptive manner, bringing one in close proximity to valuable resources (e.g. food, water, sex). However, cues can also acquire incentive motivational value (i.e. incentive salience) and come to control behavior to the point

that it becomes maladaptive. Importantly, individuals differ in the extent to which incentive salience is attributed to reward-paired cues. These individual differences can be captured using a Pavlovian conditioned approach (PCA) paradigm. When a discrete cue (lever) is repeatedly paired with delivery of food reward, some rats, termed sign-trackers attribute incentive salience to the cue; whereas others, termed goal-trackers, treat the cue only as a predictor of reward. This model, therefore, allows us to parse the incentive from the predictive properties of reward cues. Previous studies utilizing this model have shown that dopamine is critical for the attribution of incentive salience to both food- and drug-associated cues. However, the receptors involved in this form of stimulus-reward learning have yet to be identified. Here we examined the effects of dopamine D2 and D3 receptor antagonism on the expression of sign- and goal-tracking behaviors. Following PCA training, sign- and goal-tracking rats were treated with the D2/D3 antagonist raclopride (0.1 mg/kg), or the selective D3 antagonist, SB-277011A (6 or 24 mg/kg). Non-selective antagonism of D2/D3 receptors attenuated the performance sign-tracking behavior for rats that were previously classified as sign-trackers, and attenuated goal-tracking behavior in rats previously classified as goal-trackers. Interestingly, these effects were specific to the previously acquired conditioned response. In contrast, selective antagonism of D3 receptors had no effect on the expression of either the sign- or goal-tracking conditioned response for either phenotype. The present findings suggest that signaling at the dopamine D2 receptor, or perhaps some combination of synergistic activity at D2/D3 receptors, is critical for the expression of Pavlovian conditioned approach behaviors. Although previous studies demonstrated a specific role for dopamine in learning the sign-tracking response (i.e. the attribution of incentive salience to reward cues), the current findings suggest that the expression of both sign- and goal-tracking behavior can be affected when specific dopamine receptor subtypes are targeted.

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180.12/BB30. ***Chemogenetic inhibition of mesolimbic dopamine reveals excitability-dependent amphetamine action for behavior***

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Amphetamine (AMPH) is characterized as a dopamine 'releaser' as it not only blocks dopamine uptake by the dopamine transporter but also enters the presynaptic terminal, causing vesicular depletion and reverse transport of dopamine. These mechanisms are thought to be action potential-independent. Recent work using intact rats has shown that AMPH increases the frequency of phasic dopamine release events, which, in turn, is thought to be action potential-dependent and thus raises questions about the mechanisms of AMPH action. To further probe the dependency of AMPH on dopamine neuron excitability, we injected virus to deliver the Cre-dependent inhibitory (hM4D-Gi) designer receptor exclusively activated by designer drug (DREADD) into the ventral tegmental area (VTA) of transgenic rats expressing Cre recombinase under the control of the tyrosine hydroxylase promoter (TH:Cre+) and

wildtype littermates (TH:Cre<sup>-</sup>). Following transfection, systemic administration of the hM4D-Gi ligand, clozapine-N-oxide (CNO), should hyperpolarize VTA dopamine neurons, suppress excitability, and attenuate dopamine release in the NAc of TH:Cre<sup>+</sup> but not TH:Cre<sup>-</sup> rats. Using fast-scan cyclic voltammetry we found that CNO suppressed evoked dopamine release in TH:Cre<sup>+</sup> but not TH:Cre<sup>-</sup> rats thus validating our chemogenetic approach. Next, we evaluated the effect of decreased VTA dopamine excitability on AMPH-induced behavior. TH:Cre<sup>+</sup> and TH:Cre<sup>-</sup>, virus-injected rats received were pretreated with either CNO or saline and tested for AMPH-induced locomotion. A second such session was conducted with the other pretreatment in counter-balanced order. Only TH:Cre<sup>+</sup> rats exhibited significantly lower AMPH-induced locomotion following pretreatment with CNO versus saline. A separate set of locomotor tests indicated no effect of CNO on spontaneous locomotion. To examine the effects of AMPH and CNO on reward-directed behavior, TH:Cre<sup>+</sup> and TH:Cre<sup>-</sup> rats performed a rate-frequency intracranial self-stimulation task. In line with previous work, AMPH decreased the frequency threshold for self-stimulation in all animals while CNO increased threshold only in TH:Cre<sup>+</sup> rats. Both AMPH and CNO showed dose-dependency. Furthermore, pretreatment of CNO in TH:Cre<sup>+</sup> rats prevented AMPH-induced reductions in threshold. Taken together, these results demonstrate dopamine excitability-dependent behavioral actions of AMPH using a chemogenetic approach validated by electrochemical recordings thus further highlighting the importance of action potential-dependent mechanisms of this psychostimulant.

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180.16/BB34. ***Different routes of cocaine administration resolve multiple mechanisms of action to increase phasic dopamine release in the nucleus accumbens***

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Psychostimulants increase dopamine concentration in the nucleus accumbens through the blockade of the dopamine transporter (DAT). However, they also increase the frequency of dopamine release events, finding that cannot be explained by reuptake blockade alone. Rather, this effect may be mediated by systemic cocaine-induced increases in neural activity in brain regions that project to dopamine cell bodies resulting in increases in dopamine cell excitability. To further explore regionally selective actions of cocaine on phasic dopamine signaling, we administered cocaine into the lateral or fourth ventricles and compared the dopamine response to that of systemically delivered cocaine. Dopamine release in the nucleus accumbens was evoked by electrical stimulation of the ventral tegmental area and measured using fast-scan cyclic voltammetry in urethane anesthetized rats. Stimulation trains were delivered once every minutes and each train resulted in rapid and pronounced rise in dopamine followed by a rapid decay due to dopamine clearance via the DAT. The magnitude of dopamine release ([DA]<sub>max</sub>) by each stimulation train as well as the latency to decay to fifty percent of the maximum (t(1/2); index of DAT activity) were recorded. Following stable [DA]<sub>max</sub> (3 stimulations differing by less than 10%; baseline), rats received an injection of cocaine [systemic:

2.5mg/kg; lateral and fourth ventricle: 50ug in 1ul) or an equal volume of vehicle. All routes of cocaine delivery caused an increase in [DA]<sub>max</sub>. However, only systemic cocaine caused an increase in t(1/2). That hindbrain-delivered (fourth ventricle) cocaine caused an increase in [DA]<sub>max</sub> is novel. Thus, we further explored hindbrain sites that may contribute to an increase in dopamine cell excitability by using c-fos immunohistochemistry. Preliminary data suggest that fourth ventricular cocaine delivery caused a robust increase in c-fos immunoreactivity in the nucleus of the solitary tract, a region that has recently been shown to send direct projections to dopamine cell bodies. Together, the data show that cocaine induced effects on phasic dopamine signaling are mediated via actions throughout the brain including the caudal brainstem.

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180.26/BB44. ***Cannabinoid type 1 receptors facilitate conditioned reinforcement evoked by optogenetic stimulation of dopamine release***

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An ability to predict and exploit one's environment supports the rational and efficient pursuit of rewards and therefore survival. While the goals differ, reward seeking behaviors are all powerfully driven by reward-predicting cues. Mesolimbic dopamine projections from the ventral tegmental area (VTA) to nucleus accumbens (NAc) play a fundamental role in cue-directed reward seeking. Accordingly, dysregulation of this circuit is thought to promote aberrant modes of reinforcement, such as substance abuse and addictions. Alterations in cannabinoid type 1 (CB1) receptor signaling potently regulate conditioned reinforcement, such that increasing or decreasing CB1 signaling potentiates or curtails cue-directed reward seeking, respectively. While this is thought to arise from altered CB1-mediated regulation of VTA to NAc dopamine release, a direct and causal link has not been determined. We first confirmed that VTA-evoked NAc dopamine release supports positive reinforcement by demonstrating that optogenetic activation of channelrhodopsin 2 (ChR2)-expressing dopamine neurons in the VTA of DAT-Cre mice promotes vigorous intracranial self-stimulation (ICSS). Fast-scan cyclic voltammetry recordings demonstrated consistent NAc dopamine release time-locked to dopamine neuron self-stimulation. Additionally, when a predictive cue signaling reward availability was introduced, latency to lever press decreased over consecutive trials while cue-elicited NAc dopamine release increased. Thus, VTA dopamine neuronal firing sufficiently supports NAc dopamine release accompanying reward prediction. Systemic administration of the indirect CB1 receptor agonist JZL-184, which inhibits degradation of the endocannabinoid 2-arachidonoylglycerol (2AG), facilitated cue-directed ICSS as evidenced by a decrease in cue-response latency. Therefore, raising tissue levels of 2AG facilitates behaviors reinforced exclusively by VTA-evoked dopamine release. In separate experiments, we confirmed that blocking CB1 receptors with AM-251 inhibited cue-evoked NAc dopamine release and reward seeking in a food-reinforced task. Importantly, pairing cue presentation with optical stimulation of VTA dopamine neurons reversed the effects CB1 blockade, suggesting that deficits in cue-directed

reward seeking arise exclusively from CB1 receptor antagonists suppressing VTA-evoked dopamine release. Collectively, this work confirms and refines current understanding of the canonical role of CB1 receptor signaling in VTA-evoked NAc dopamine release and conditioned reinforcement.

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180.27/BB45. ***Enhanced extinction of cocaine conditioned place preference via glutamate transporter activation is associated with reduced nucleus accumbens c-Fos expression***

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Ceftriaxone is a  $\beta$ -lactam antibiotic that activates glutamate transporter subtype (GLT-1), Clavulanic acid is structurally-related  $\beta$ -lactamase inhibitor that retains the  $\beta$ -lactam core required for glutamate transporter activity but displays more patient-friendly characteristics such as enhanced brain penetrability, minimal antibacterial activity, and oral activity. We have previously shown that both drugs attenuate the rewarding aspects of cocaine by enhancing extinction from cocaine conditioned place preference (CPP). C-Fos is proto-oncogene that is expressed within neurons following depolarization. The current study was an attempt to correlate these behavioral effects with neurochemical changes by evaluating nucleus accumbens c-Fos protein expression. Animals that underwent a 5-day extinction period following cocaine CPP during which ceftriaxone, clavulanic acid or saline was administered daily were challenged with a final cocaine exposure in their drug-paired environment before being euthanized. Brains were sectioned and processed for c-Fos immunohistochemistry. Results indicate that c-Fos expression in the shell and the core of the nucleus accumbens was reduced significantly in animals that received either ceftriaxone or clavulanic acid during extinction from cocaine CPP. These findings suggest that pharmacologic enhancement of glutamate clearance disrupts the in vivo actions of cocaine which are correlated with reduced expression of a proto-oncogene marker of neuronal activity.

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287.07. ***Reappraisal alters the construction the emotional experiences***

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Successful regulation of emotions is important for maintaining our mental and physical health. Though many studies have reliably demonstrated that strategies such as reappraisal are effective in decreasing negative affective states, the precise mechanism of how this process unfolds remains unclear. One popular account argues that reappraisal directly attenuates the affective experience by recruiting executive control systems. Alternatively, reappraisal may not directly decrease the brain's reactivity to negative stimuli, but instead qualitatively changes the affective experience. Here we develop a sensitive

and specific signature of negative affect elicited via negative arousing images and evaluate these competing hypotheses. We find that participants report lower negative affect ratings when instructed to reappraise, but interestingly, our negative affect signature systematically overestimates how people are feeling. Region of interest analyses suggest that this may be a consequence of processes in medial and lateral prefrontal cortex that are likely shared in both emotion generation and reappraisal, but are amplified during reappraisal. We used whole-brain multivariate moderation analysis to test this hypothesis and find that the negative affect signature is reconfigured when reappraising compared to naturally reacting to negative stimuli. This is evidenced by increased weights in the dACC and decreased weights in the MPFC, left amygdala, and areas of the visual cortex. In addition, we identify multivariate representation of reappraisal that can discriminate between reappraisal and reactive states with 91% accuracy in leave-one-subject out cross-validation. The most predictive weights of this reappraisal detector partially overlap with the moderation analysis (e.g., dACC) suggesting that cognitive reappraisal processes contribute to the change in emotional experience. Together, these results suggest that reappraisal recruits distinct neural circuitry from emotional reactivity and appears to alter the construction of the emotion experience.

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#### 287.08. *Brain mechanisms of worse than expected rewards*

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This talk will focus on the brain mechanisms involved in learning about unexpected omissions of reward, and outline a model of interactions between those regions. This account is distinct from traditional reinforcement learning theories, which treat both worse than expected and better than expected rewards similarly as differences in prediction error. However, we propose that learning about reward omissions draws on brain circuitry involved in negative valence and stress, in particular the lateral habenula, which drives dopamine dips for negative valence and reward omissions. Additionally, we will describe a computational model based on circuit-level research from animal models that proposes specific interactions between limbic brain regions such as the ventral striatum, amygdala, and lateral habenula in positive and negative valence conditioning tasks. As a test of these model predictions, we ran a conditioned inhibition fMRI study, where a CS paired with a juice reward was later paired with a Inhibitor, that caused omission of the expected juice reward. This conditioned inhibition procedure has previously been tested in monkey studies (Tobler, 2003), which have found that dopamine neurons respond with a dip to the Inhibitor that predicts a reward omission. Based on recent research showing that the lateral habenula plays an important role in causing dopamine dips, we predicted that the habenula would play a role in driving reduced VTA activity for the Inhibitor. In this study, we observed significantly more activity in the SN/VTA for the CS+ than the CS- ( $p = .044$ ,  $t(1,18) = 2.16$ ). Based on this result, we ran a whole brain functional connectivity analysis to look for brain regions where correlations with single-trial VTA estimates in each of our conditions varied across the different CS types. We found that lateral habenula activity was negatively correlated with activity in the VTA for the Inhibitor, but not

the other CS types, supporting our model of the role that lateral habenula plays in signaling the VTA for predictors of reward omission. Additionally, I will discuss other predictions the computational model makes about the involvement of the amygdala and ventral striatum in conditioned inhibition and how they are borne out by the data.

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**287.12. *Coping with setbacks: Emphasis on learning from setbacks counteracts effects of acute stress on persistence***

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The experience of a negative outcome while striving for a goal can cause negative affect, and requires one to cope with this setback in order to persist with the goal. For example, a negative performance evaluation may cause negative affect for an employee trying to earn a promotion. That setback may be exceptionally difficult to cope with if the employee is already experiencing stress from a situation at home. However, setbacks can also provide useful information to improve efforts to achieve a goal. For example, a negative evaluation can guide an employee's future performance towards a goal. In this study, we address two questions. First, we investigate how preexisting stress influences the ability to cope with setbacks. Second, we probe whether emphasizing the informative aspects of setbacks can promote better persistence with goals. Human participants played a game in which they encountered setbacks and made decisions to persist or give up on a goal after the setbacks. Increasing the informative properties of setbacks promoted better persistence after setbacks and diminished negative affect. Prior exposure to an acute cold water stressor (which elicited an increase in salivary cortisol) had a deleterious influence on such persistence behavior. Importantly, the decrease in persistence due to prior stress (compared to a control group) was only observed when uninformative (i.e., random) aspects of the setbacks were emphasized. When informative properties of the setbacks were emphasized, persistence deficits were not observed in the stress group. This is consistent with prior neuroimaging data (Bhanji & Delgado, 2014) demonstrating dissociable neural mechanisms underlying participants' responses to setbacks. Ventral striatal signal decreases were associated with using information from setbacks to correct mistakes and predicted greater persistence through the setbacks. Ventromedial prefrontal cortex (vmPFC) signal increases predicted persistence when setbacks were uninformative (i.e., random), and vmPFC signal changes mediated the relationship between increased negative affect and persistence behavior. Together these results suggest that preexisting stress can impair the ability to cope with negative affect elicited by setbacks, a process potentially associated with vmPFC function. However, coping with setbacks as a learning opportunity, which may rely on striatal mechanisms, can counter the effects of stress.

**313.01/I26. *The effects of smoking reduced nicotine cigarettes upon resting state functional connectivity, craving and withdrawal in young smokers***

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Smoking contributes to more than 540,000 premature deaths each year in the United States, and enacting policy to reduce the nicotine content is viewed as a way to reduce cigarette use. This study examined whether the nicotine yield of cigarette affects the response to smoking, measured as functional coupling between brain networks that have been shown to be related to cigarette craving, withdrawal and cognition - the executive control network (ECN), default mode network (DMN), and the salience network (SN) - along with behavioural measures of craving, withdrawal, and sustained attention. Fifteen participants (11 men, 4 women), 18-25 years of age, who smoked cigarettes daily, each completed testing on 4 days in two sessions: before and after the first cigarette of the day (after overnight abstinence). Testing included resting state fMRI scans, questionnaire measures including the Urge To Smoke (UTS) scale and the Shiffman-Jarvik Withdrawal (SJW) scale, and the Rapid Visual Information Processing (RVIP) task, a test of sustained attention. Participants smoked a research cigarette containing one of four nicotine yields (0.027, 0.110, 0.231 or 0.763 mg), or their own preferred-brand. Independent Component Analyses of the fMRI data revealed 3 ECN networks, 5 DMN networks, and 1 SN network. Positive coupling between ECN and DMN networks and SN and DMN networks was reduced after smoking, with reduction in a greater number of networks after smoking the participants' preferred brand than the .027 mg cigarette. Smoking also enhanced sustained attention, as measured via RVIP hits and A' scores, with greater improvements in A' scores after smoking cigarettes with higher nicotine yields. Finally, smoking reduced cigarette craving and withdrawal, irrespective of the type of cigarette smoked or the nicotine yield. These results extend previous observations that both ECN-DMN coupling and SN-DMN coupling are reduced following cigarette smoking, by showing that these changes depend upon the nicotine yield of the cigarette. The results also suggest that improvements in sustained attention depend on the nicotine content of a cigarette, but that self-reported smoking-induced reductions in craving and withdrawal do not. The results suggest that RSFC may be a more sensitive index of response to smoking than subjective self-reports of craving.

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**313.04/I29. *The effect of nicotine administration and withdrawal on sleep in mice***

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Sleep disturbances are commonly reported symptom during tobacco cessation attempts. They are currently the only symptom of human nicotine withdrawal syndrome that has not been correlated in a

rodent model. The current study investigates the effect of nicotine administration and withdrawal on sleep quantity and quality in a forced oral nicotine mouse model. Nine subjects were implanted with EEG and EMG recording devices using standard procedures. After recovery and acclimation period, data was recorded continuously for a 4-week period, certain days were chosen over each condition for sleep scoring. Mice had ad libitum access to food and a drinking water solution containing .2% saccharin. Baseline sleep and wake data was scored for three consecutive 24 periods, and subsequently averaged. Immediately following baseline, five of the subjects began receiving 200µg/ml of nicotine for a period of weeks (nicotine group). The control group did not experience any changes. Data for this condition was scored on days 1, 4, 8, 11, and 13. Withdrawal was precipitated spontaneously by excluding the nicotine from the drinking solution; the first two days of withdrawal were scored. Nicotine consumption tended to decrease total sleep. The effect was primarily seen during the lights off period and can mostly be explained by decrease in time spent in NREM. Additionally, nicotine withdrawal appears to have an effect on the number of stage changes, both the number of awakenings from sleep and the number of total stage changes. The current data suggests of effect of nicotine consumption and withdrawal on the sleep wake cycle.

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313.07/132. ***Activation of the dynorphin-kappa system in the central nucleus of the amygdala mediates the negative emotional state of nicotine withdrawal but not escalation of nicotine intake***

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Abstinence from nicotine often results in emergence of a negative emotional state that predict relapse and escalation of nicotine intake in humans and rats. Prodynorphin and activation of kappa receptors have been shown to produce withdrawal-like symptoms, suggesting that activation of the dynorphin-kappa system may be responsible for the emergence of a negative emotional state leading to escalation of nicotine intake. However, the causal role of activation of the dynorphin-kappa system on measures of negative emotional states and nicotine intake in dependent animals remains to be demonstrated. To test this hypothesis we tested the effect of systemic blockade of kappa receptors using a long-lasting kappa antagonist (nor-BNI), and downregulation of prodynorphin in the central nucleus of the amygdala using a viral vector (AAV-shPdyn) on withdrawal induced-pain, -conditioned place aversion, escalation of nicotine intake and stress-induced reinstatement. We found that withdrawal-induced hyperalgesia, conditioned place aversion to withdrawal and nicotine escalation, were prevented by nor-BNI (30 mg/kg). Immunohistochemical analysis showed that prodynorphin's content was increased in the CeA in nicotine dependent rats, but not in non-dependent rats. Downregulation of prodynorphin in the CeA using AAV-shPdyn did not affect nicotine escalation, but significantly decreased withdrawal-induced hyperalgesia, aversion to withdrawal and stress-induced reinstatement using the pharmacological stressor Yohimbine (1.25mg/kg). These results demonstrate that while activation of kappa receptors mediates both the negative emotional state of withdrawal and the increased motivation for nicotine

after abstinence, increased prodynorphin levels in the CeA only mediates the negative emotional state of nicotine withdrawal, but does not affect the motivation for nicotine. This report provides preclinical evidence for the efficacy of kappa antagonists in reducing the motivational effects of nicotine withdrawal, and identify that upregulation of prodynorphin in the CeA is responsible for the emergence of the negative emotional state of nicotine withdrawal.

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314.12/J8. ***Ventral subiculum is critical for context-induced relapse to alcohol seeking after punishment-imposed abstinence***

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Background: Alcoholics typically abstain because of negative consequences associated with excessive drinking and exposure to contexts previously associated with alcohol use often triggers relapse. We recently developed a rat model that captures some characteristics of this human condition: exposure to the alcohol self-administration environment (context A) after punishment-induced suppression of alcohol seeking in different environment (context B) provokes relapse to alcohol seeking in alcohol-preferring P-rats. Here, we studied the role of ventral subiculum (vSub) and projections from vSub to nucleus accumbens (NAc) shell in this form of relapse. Methods: We first assessed the effect of reversible inactivation of vSub by GABA<sub>A</sub>+GABA<sub>B</sub> receptor agonists (muscimol+baclofen) on context-induced relapse to alcohol seeking. We then assessed neuronal activity associated with context-induced relapse by measuring Fos, a marker of neuronal activity. We combined Fos with the retrograde tracer cholera toxin subunit B (CTb, injected into NAc shell), to assess activation in neurons projecting to NAc shell. We assessed activation in glutamatergic inputs to NAc shell, including vSub, ventral medial prefrontal cortex (vmPFC), paraventricular thalamus (PVT), or basolateral amygdala (BLA). Results: Muscimol+baclofen injections into vSub decreased context-induced relapse after suppression of alcohol seeking by punishment. Double-labeling analysis of Fos+CTb demonstrated that context-induced relapse was associated with selective activation of NAc shell projecting neurons in vSub but not vmPFC, PVT, or BLA. Conclusion: These results demonstrate critical role of vSub in context-induced relapse to alcohol seeking after punishment and suggest that vSub may promote alcohol seeking during relapse by activation of NAc shell.

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314.13/J9. ***NMDA and GABAA receptor-mediated plasticity in the ventral tegmental area by acute and chronic ethanol***

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Ventral tegmental area (VTA) GABA neurons are important substrates for alcohol effects in the mesolimbic dopamine (DA) system originating in the VTA and projecting to the nucleus accumbens (NAc). We have previously reported that VTA GABA neurons are sensitive to ethanol at physiologically-relevant doses, exhibit tolerance to acute ethanol, and evince marked hyperactivity during withdrawal from chronic ethanol, which may explain the deficits in DA transmission associated with alcohol dependence. We evaluated glutamate (GLU) NMDA and GABAA receptor-mediated synaptic transmission to VTA GABA neurons during withdrawal from acute and chronic ethanol. To accomplish these studies, we used standard whole-cell and cell-attached mode electrophysiological techniques to evaluate VTA GABA neuron responses in CD-1 GAD GFP mice. In naïve animals, withdrawal from an in vivo 24 hr intoxicating dose of ethanol (4 g/kg), enhanced the AMPA/NMDA ratio in VTA GABA neurons. In animals made dependent on ethanol by twice daily injections of 2.5 g/kg ethanol for 6 days, there was no change in the AMPA/NMDA ratio. Similar findings were obtained when animals were exposed to chronic intermittent ethanol (CIE) in alcohol vapor chambers, where they were exposed to 250 mg% blood alcohol levels for 12 hours/day during their dark cycle for 3 weeks. High frequency stimulation induced long-term depression (LTD) of evoked GABAA receptor-mediated IPSCs in VTA GABA neurons in air-exposed animals. Work is in progress to evaluate LTD(GABA) in mice exposed to CIE. These findings suggest that GLU NMDA receptor-mediated plasticity accompanies withdrawal from a single exposure to ethanol, but GABAA receptor-mediated plasticity is operational during withdrawal from chronic exposure to ethanol, suggesting that a switch occurs in GABAA receptor inhibition similar to what has been shown during opiate dependence. These findings have important implications for understanding the adaptations in the mesolimbic reward pathway along the continuum to alcohol dependence.

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314.14/J10. ***Functional switch in GABA(A) receptors on VTA GABA neurons by acute and chronic ethanol***

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The motivational effects of opiates and ethanol switch from dopamine (DA)-independent to a DA-dependent pathway during drug dependence. A corresponding change occurs in ventral tegmental area (VTA) GABA(A) receptors in opiate-dependent animals, which switch from a GABA-induced hyperpolarization of VTA GABA neurons to a GABA-induced depolarization. This effect occurs due to increased BDNF expression and corresponding activation of the trkB receptor on VTA GABA neurons. The aim of this study was to evaluate VTA GABA neuron excitability and GABA synaptic transmission to VTA GABA neurons under ethanol-naïve, acute and dependent conditions. To accomplish these studies, we used standard whole-cell and cell-attached mode electrophysiological techniques to evaluate acute and chronic ethanol effects on VTA GABA neurons in CD-1 GAD GFP mice. In saline-injected controls, superfusion of the GABA(A) receptor agonist muscimol (IC50 = 100 nM) decreased VTA GABA neuron firing rate in a dose-dependent manner. In animals given a single, in vivo 24 hr intoxicating dose of ethanol (4.0 g/kg), VTA GABA neuron firing rate was relatively resistant to the effects of muscimol.

Similar findings were seen in mice made dependent on ethanol by twice daily injections of 3.0 g/kg ethanol for two weeks or chronic intermittent ethanol (CIE) vapor exposure (200 mg% BAL) for 2-3 weeks. BDNF expression was increased in both the VTA and the nucleus accumbens (NAc) during withdrawal from CIE. Work is in progress to evaluate the effects of blocking the trkB receptor on alcohol dependence and VTA GABA activity. These findings suggest that VTA GABA neurons undergo a switch in GABA(A) receptor function in ethanol-dependent animals, similar to opiate-dependent animals. There also appears to be some GABA(A) plasticity after an acute administration of ethanol. We suggest these changes occur through BDNF activation of the trkB receptor.

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### 315.01/J11. *Estrogen-potentiated reinstatement of cocaine seeking*

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Although it is generally accepted that peak physiological levels of the ovarian hormone estrogen confer enhanced relapse vulnerability in female cocaine addicts, the underlying mechanisms are not yet well understood. To investigate this, we sought to determine whether the primary estrogen 17 $\beta$ -estradiol (E2), like the stress hormone corticosterone, can promote reinstatement of cocaine seeking in response to a “subthreshold” dose of cocaine that is otherwise insufficient for reinstatement. Sexually mature female Sprague Dawley rats (90 days old/280g minimum at study onset) were surgically implanted with intravenous catheters and underwent short access (2 hour) cocaine self-administration (0.5mg/kg/0.2mL i.v. infusion) for 14 days prior to extinction training. To avoid effects on self-administration and extinction and isolate effects on reinstatement and related responses, rats did not undergo surgical ovariectomy (OVX) until after reaching extinction criterion (<15 lever presses/2-hour session for 2 consecutive days). After allowing 7 days to recover and ensuring that responding still met extinction criterion, a counterbalanced design was used to administer the following tests to each rat: E2 (10ug/kg, i.p.; 1hr pretreatment) + saline, vehicle + saline, E2 + cocaine (various doses, i.p.), vehicle + cocaine. We determined that, under these conditions, both 0.625mg/kg and 1.25mg/kg cocaine were subthreshold doses, while 2.5mg/kg cocaine was a supra-threshold dose. Although pretreatment with E2 had no effect on 0.625mg/kg cocaine, potentiated reinstatement was seen with E2 + 1.25mg/kg cocaine. Furthermore, E<sub>2</sub> potentiated responding to 2.5mg/kg cocaine, suggesting synergistic effect between the hormone and psychostimulant. Although further testing is required to determine if higher physiologically-relevant doses of E2 may potentiate reinstatement, these results indicate that we have developed a model to study how estrogen may set the stage for relapse in female cocaine addicts. Further investigations into localized mechanisms are currently underway.

**315.02/J12. *Role of a crf receptor-regulated dopaminergic projection from the ventral tegmental area to the prelimbic cortex in stress-induced relapse***

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Relapse to cocaine use is frequently precipitated by stress. It is well accepted that mesocortical dopamine (DA) neurons originating from the ventral tegmental area (VTA) and projecting into the prelimbic cortex (PL) are involved in drug-seeking behavior. However, the neural mechanism through which stress regulates VTA neurons remains elusive. We and others have implicated the neuropeptide corticotropin releasing factor (CRF) in stress-induced relapse. We hypothesize that CRF release into the VTA during stress activates a subset of VTA DA neurons that project into the PL, resulting in DA D1 receptor activation and, reinstatement of drug-seeking behavior. Here we show that electric footshock (EFS)-induced reinstatement is associated with an increase in PL Fos expression and is observed in animals with history of long access (LgA; 6hr) but not short-access (ShA; 2hr) self-administration (SA). This is consistent with our previous finding that stress-induced reinstatement is dependent on history of LgA. In addition, using the retrograde tracer, cholera toxin b, we show that VTA cells that project into the PL are active during stress-induced reinstatement. Stress-dependent PL Fos activity is, in part, due to the activation of VTA CRFR1. Antagonism of CRFR1 with intra VTA antalarmin (250ng) prevented the increase in Fos expression within the PL and EFS-induced reinstatement of cocaine seeking. We also examined the role of VTA CRFR1-mediated activation of DA projections to the PL and, thereby, D1 receptor activation in the PL, in stress-induced reinstatement using a disconnection approach involving unilateral intra-VTA delivery of the CRFR1 antagonist, antalarmin and contralateral intra-PL injection of the D1 receptor antagonist, SCH 23390 (200ng). Disconnection of the PL-VTA pathway blocked stress-induced reinstatement of cocaine seeking. Ipsilateral control injections failed to block EFS-induced reinstatement. Thus, the increase in PL Fos is dependent on both a history of LgA SA, and activation of CRFR1 within the VTA, consistent with our previous report that reinstatement to intra-CRF (300ng) VTA is heightened following LgA SA. Heightened CRF-mediated drug seeking and activation of the mesocortical pathway following LgA SA is likely attributable to increased VTA CRFR1 expression, as VTA CRFR1 mRNA expression measured using in situ hybridization is increased in LgA rats relative to ShA rats and saline controls. Our results suggest that VTA CRFR1 activation induces relapse to cocaine use by activating DA cells that project to the PL and that CRFR1 regulation of this pathway is heightened as a result of prior cocaine use.

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**315.03/J13. *Corticosterone potentiates reinstatement of cocaine seeking through endocannabinoid-mediated inhibition of GABAergic neurotransmission in the prelimbic cortex***

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Stress is a powerful trigger for relapse and can not only induce relapse but can potentiate the response to other triggers for drug use. We have shown that under certain self-administration conditions, stress alone does not reinstate cocaine-seeking. However a stressor, electric footshock stress (EFS), can potentiate reinstatement when paired with low dose cocaine. This effect is corticosterone-dependent and the effect of EFS is mimicked by systemic or intra-prelimbic cortex (PL) corticosterone indicating that it is not only necessary but sufficient and the PL, a region critical for reinstatement, is a site of action. Exactly how corticosterone potentiates reinstatement is not fully understood but may involve interactions with the endocannabinoid (eCB) system. Stress increases eCB production in the medial prefrontal cortex, and, like stress-potentiated reinstatement, is glucocorticoid-dependent and we have shown systemically that eCB signaling mediates stress-potentiated reinstatement. The present study examined this effect further by assessing the potential mechanism of corticosterone action in the PL in this effect. Male SD rats self-administered cocaine (0.5 mg/kg/inf; 1 x hrs/day) and then underwent extinction training followed by reinstatement tests. EFS paired with low-dose cocaine- (2.5 mg/kg, ip) induced reinstatement whereas either low dose cocaine or EFS alone did not. Intra-PL infusions of the cannabinoid receptor 1 (CB1R) antagonist, AM251 (0.3 µg) given 15 min prior to reinstatement tests blocked both EFS- and corticosterone-potentiated reinstatement suggesting that these effects are mediated through eCB signaling in the PL. In addition, the effect of EFS or corticosterone can be mimicked by an intra-PL infusion of the CB1R agonist, WIN 55,212 (50 ng), suggesting that eCB signaling in the PL is not only necessary but sufficient for this effect. The contribution of specific eCBs to potentiated reinstatement is currently being tested. Altogether, these data suggest that glucocorticoid-endocannabinoid interactions in the PL mediate stress-potentiated reinstatement. As CB1Rs are located on GABAergic interneurons in the PL, corticosterone effects may be the result of eCB-mediated inhibition of GABA. In support of this, bath application of corticosterone to PL slices inhibits GABAergic neurotransmission in a CB1R-dependent manner. The involvement of intra-PL GABAergic signaling in corticosterone-potentiated reinstatement is currently being examined. These findings support the hypothesis that corticosterone acts in the PL, through eCB-mediated inhibition of GABA, to potentiate reinstatement of cocaine seeking.

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315.05/J15. ***history of physical, emotional, or sexual abuse predicts higher mesolimbic response to drug cues in cocaine-dependent patients***

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Aims: Previous studies have reported that a history of adverse experiences is associated with higher rates of mental health issues, including addiction. Here, we investigated whether a history of physical,

emotional, or sexual abuse in cocaine-dependent patients was associated with greater cocaine cue-triggered activity in nodes of the mesolimbic reward circuitry. **Methods:** Treatment-seeking cocaine-dependent patients (n=26) were recruited as part of a 6 month treatment study. Before the start of the study, participants were administered the Addiction Severity Index (ASI), which contains questions measuring prior abuse (emotional, physical, or sexual). After inpatient stabilization, participants were scanned with event-related blood-oxygen-level-dependent functional MRI during exposure to brief (500 msec) evocative (cocaine, sexual, aversive) vs. neutral cues. Forty-eight images of each cue type were presented in a quasi-random order. Imaging preprocessing (alignment, registration, normalization, smoothing, and motion correction) and first level analysis were conducted within a standard SPM8 pipeline. The responses to the abuse questions were used to create a split (abuse yes - abuse no) for the cocaine-neutral cue contrast. **Results:** From the ASI, there were 12 patients that reported a history of abuse and 1 that reported no abuse. As predicted, patients reporting abuse had greater brain activation to the cocaine (vs. neutral) cues in several mesolimbic regions, including the midbrain (VTA), ventral striatum, dorsal striatum, and caudal orbitofrontal cortex to drug cues compared to patients reporting no abuse ( $2 < t < 5$ ). **Conclusions:** Individuals with adverse life events have been found to be more susceptible to drug addiction. In our study, even though all patients were cocaine dependent, our results provide initial evidence that a history of abuse could have a brain impact (i.e., heightened limbic response to drug cues) that drives drug seeking. Our results highlight heterogeneity within a cocaine-dependent population, indicating the need for individually-tailored treatment. Importantly, to our knowledge, this is the first evidence of a history of abuse on brain vulnerability that is linked to relapse.

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315.06/J16. ***Corticotropin releasing factor and dopamine interactions in heterogeneous ventral tegmental area: How can aversive experiences heighten cocaine self-administration?***

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Intermittent social defeat (on days 1, 4, 7, and 10) escalates later cocaine self-administration. Both rewarding and stressful stimuli increase extracellular dopamine (DA) in ventral tegmental area (VTA) projection targets—the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc). Corticotropin releasing factor (CRF) and its receptors (CRFR1 and CRFR2) are located in the VTA and may influence DA activity during stress, resulting in later heightened vulnerability to addiction. These experiments explore how CRF acts on its receptors both during and after stress to influence mesocorticolimbic DA and cocaine self-administration. In vivo microdialysis on d 1 and 10 of social defeat showed phasic CRF release in the VTA. In the rostral VTA, CRF was phasically increased on d 10, but not d 1. In the caudal VTA, CRF was phasically increased on both d 1 and d 10, with greater increase on d 1. Additionally, baseline CRF concentration was increased on d 10 compared to d 1 regardless of probe placement. This CRF release in the VTA affected extracellular DA in both the mPFC and NAc, mediated by CRFR2. Rats

were microinjected with a CRFR1 or CRFR2 antagonist into the VTA prior to each defeat, and microdialysis for DA in the mPFC and NAc conducted concurrently on d 1 and 10. Extracellular DA was significantly increased in both regions during both acute and repeated social defeat. On d 1, intra-VTA CRFR2 antagonism prevented the DA increase in the NAc, while not affecting mPFC DA. CRFR1 antagonism had no effect. On d 10, intra-VTA CRFR2 antagonism prevented the DA increase in both the mPFC and NAc, while CRFR1 antagonism still had no effect. Intra-VTA antagonism of both CRFR1 and CRFR2 during each defeat prevented later escalated cocaine self-administration during 2 h “binge”. CRFR1 antagonism in the caudal, but not rostral, VTA prevented escalated “binge” cocaine self-administration in stressed rats, while the converse was found for CRFR2 antagonism. VTA CRF also played a role in later cocaine seeking. Rats acquired cocaine self-administration and were placed in forced abstinence for 15 d, followed by context-induced reinstatement testing. Previously stressed rats pressed the previous active lever significantly more than controls, which was blocked by both intra-VTA CRFR1 and CRFR2 antagonism. In vivo microdialysis found a phasic increase in CRF during reinstatement, although tonic levels were significantly higher in previously stressed rats compared to controls. In conclusion, CRF in the VTA plays a key role in DA function during stress, promoting neuroadaptations driving later increased drug taking and seeking.

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315.07/J17. ***Identification of a novel CRF-VTA microcircuit in the mouse midbrain underlying stress and psychostimulant sensitization***

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Corticotropin releasing factor (CRF) signaling in the ventral tegmental area (VTA) regulates stress-induced psychostimulant self-administration. However, the source of VTA-CRF and the molecular mechanisms underlying this drug-seeking behavior remain unclear. Thus, we used viral-vector-based tract-tracing by stereotaxically infusing an AAV-Flex-ChR2 virus bilaterally in the ventral-posterior-medial (VPM) region of the VTA in CRF-Cre mice. CRF neurons in the lateral hypothalamus (LH) and dorsal raphe nucleus (DRN) projected to and from the paranigral (PN) and parainterfascicular (PIF) subnuclei of the VTA. DRN-VPM CRF dendrites form putative synapses on subsets of dopaminergic (DA-ergic) neurons co-expressing CRF1/2 receptors in the VPM while LH-VPM CRF processes did not. Both of these circuits were activated, evident by c-FOS-immunoreactivity (-ir), following a single 30-minute restraint or 15-minute social defeat stress. Although this circuit is activated by chronic stress (i.e., 10 days of social defeat), there are fewer synapses double-labeled with PSD95 and VGLUT1. Thirty-minutes of restraint stress increased CRF-ir fibers in the PN/PIF, consistent with microdialysis studies demonstrating CRF release in the VPM of intruder rats experiencing social defeat stress. Specifically activating the PN/PIF using either optogenetics or Gq-DREADD in CRF-Cre mice was sufficient to mimic chronic social defeat stress-induced drug-seeking behavior. Daily 30-minute activation of CRF signaling in the PN/PIF for 10 consecutive days followed by 10-days of rest induced behavioral sensitization to a single intra-peritoneal injection of dextro-amphetamine (d-AMPH, 1.5mg/kg). Together, these data suggest that CRF neurons in

the DRN are activated by stress, releasing CRF in the PN/PIF, which modulates behavioral sensitization to d-AMPH. This research was supported by an NIMH R01 research grant NS073574 to J.M., a NIDA grant 03173 to K.M., and the Tufts Center for Neuroscience Research grant P30 NS047243. The authors declare no biomedical, financial, or potential conflicts of interest.

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**315.08/J18. *Prevention of escalated cocaine self-administration and cocaine seeking by CRF R1 antagonist in socially defeated mice***

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Social defeat stress can result in escalated cocaine self-administration and cocaine seeking in rodents. Corticotrophin releasing factor (CRF) represents the initial step in the stress cascade that leads to escalation of drug use and drug seeking behaviors. However, the contribution of CRF receptor subtypes to cocaine reinstatement and their role during different phases of stress-induced cocaine self-administration still remains to be defined. The current study examines the effects of CRF R1 antagonist on escalated intravenous cocaine self-administration and cocaine reinstatement as a result of exposure to social defeat stress in mice. (1) The CRF R1 antagonism (CP 376,395, 15mg/kg) given 30 min prior to each social defeat episode prevented stress-induced cocaine self-administration in mice. (2) Administration of CP 376,395 (5 mg/kg and 15 mg/kg) 10 days after the last episode of social stress, dose-dependently reversed the escalation of cocaine intake. (3) In addition, we showed that CP 376,395 administration prior to reinstatement test decreased stress-induced cocaine seeking behaviors. (4) To further explore the role of CRF R1 in specific brain sites, CP 376,395 (0.5 µg/ 0.2 µl and 1 µg/ 0.2 µl) was delivered directly into the ventral tegmental area (VTA) before cocaine self-administration session 10 days after the last stress episode. Intra-VTA antagonism of CRF R1 was sufficient to reverse stress-induced escalated cocaine self-administration. These findings suggest that CRF R1 exerts multiple roles during initial reaction to social stress and in long-term neuroadaptations that are relevant to escalated cocaine self-administration and cocaine seeking, providing a potential target for therapeutic interventions for stress-induced drug use disorders.

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**315.09/J19. *Increased sensitivity to cocaine-induced relapse to drug-seeking behavior in organic cation transporter 3 knockout mice***

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Cocaine addicts report that craving responses to drug-associated stimuli are intensified during periods of stress, resulting in heightened susceptibility to relapse of drug use. These reports are paralleled by

findings that stress can potentiate the reinstatement of cocaine-seeking behavior by drug-associated cues in rodents. Together, these studies suggest that stress may act as a “stage-setter”, inducing state-dependent changes in the sensitivity of brain reward circuits to the reinforcing properties of drugs, and enhancing the potency of drugs of abuse or drug-associated cues to induce relapse. Thus, interactions between pathways activated by stress and by cocaine-associated stimuli are likely to be critical determinants of relapse vulnerability. However, the mechanisms underlying these interactions are not completely understood. We recently demonstrated that corticosterone (CORT) acutely blocks dopamine clearance in the nucleus accumbens (NAc), likely mediated by the uptake2 transporter organic cation transporter 3 (OCT3), a high-capacity corticosteroid-sensitive monoamine transporter. We provided evidence that, through this mechanism, CORT potentiates the actions of cocaine on dopamine signaling and reinstatement of drug-seeking behavior in rats. We have hypothesized that decreased clearance of dopamine in the NAc, due to CORT-induced inhibition of OCT3, enhances dopaminergic neurotransmission, resulting in increased sensitivity to natural and cocaine reward, and heightened vulnerability to relapse of cocaine-seeking behavior. While OCT3 is the most likely mechanism underlying CORT effects on dopamine clearance and relapse, support for its role in these processes is based on pharmacological tools (CORT and normetanephrine) which can exert actions at other, non-OCT3, targets. To more definitively test the hypothesis that OCT3 inhibition underlies CORT-induced potentiation of relapse, the present studies examined the effects of CORT and normetanephrine, two inhibitors of OCT3, on cocaine-primed reinstatement of conditioned place preference in wild type and OCT3 knockout mice. Compared to wild-type mice, OCT3-knockout mice were more sensitive to low-dose cocaine-induced reinstatement of conditioned place preference. In wild-type mice, both CORT and normetanephrine potentiated low-dose cocaine-induced potentiation of CPP, while both OCT3 inhibitors were without effect in OCT3 knockout mice. These studies suggest that the previously-described effects of CORT on relapse to drug seeking behavior are mediated, at least in part, by inhibition of OCT3-mediated dopamine transport.

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315.10/J20. ***Corticosterone potentiates the effect of cocaine on nucleus accumbens dopamine release and clearance***

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Stress and cocaine act synergistically to increase extracellular dopamine and drive cocaine seeking. This interaction is mediated in part by the effects of corticosterone in brain areas that receive dopamine innervation, such as the nucleus accumbens and medial prefrontal cortex. We have recently demonstrated that corticosterone acts to decrease dopamine clearance in the nucleus accumbens via a dopamine transporter (DAT)-independent mechanism likely involving the inhibition of organic cation transporter 3 (OCT3)-mediated transport. We have also demonstrated that corticosterone potentiates the effects of cocaine on nucleus accumbens dopamine signaling and drug-seeking behavior. However, it

is not known whether the corticosterone-induced reduction in clearance is accompanied by an increase in dopamine release events, or whether corticosterone affects clearance in the absence of DAT blockade. We examined the effect of acute corticosterone treatment on dopamine release and clearance in the nucleus accumbens core and shell of behaving rats to examine how the stress hormone acts centrally to augment the dopamine response to cocaine. Using fast scan-cyclic voltammetry, we measured naturally-occurring transient dopamine release events during a baseline period, after a systemic injection of corticosterone (2 mg/Kg, ip) or vehicle (2.5% EtOH, ip), and after a subsequent systemic injection of low-dose cocaine (2.5 mg/Kg, ip). Results show that corticosterone potentiates cocaine's effect on dopamine transient frequency and amplitude, suggesting that the reduction in dopamine clearance increases the number of spontaneous release events. Furthermore, preliminary findings indicate that corticosterone alone produces a mild increase in extracellular dopamine, suggesting that the inhibition of OCT3 modulates dopamine signaling in the absence of DAT blockade. Ongoing studies are investigating the potential impact of corticosterone on phasic dopamine encoding of natural rewards in order to determine whether the effects of corticosterone extend beyond situations in which the DAT is blocked by cocaine.

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### 315.12/J22. *Cannabinoid exposure in adolescence modulates cocaine reward in adulthood*

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Marijuana is the most commonly abused illicit drug among adolescents, and regular use of cannabinoids (CBs) in this vulnerable population is associated with the development of psychiatric disease including drug abuse. Human research indicates that adolescent CB use predicts future cocaine (COC) abuse, however, the underlying cause of this phenomenon is unclear. Adolescent CB exposure disrupts the dopaminergic response to COC in adulthood suggesting that CB use may disturb the subjective experience of COC. It is well documented that immediate euphoria produced by COC administration is subsequently replaced by feelings of dysphoria and anxiety. Thus, it is likely that an individual's experience of either of these opposing processes may motivate successive COC use. Here we utilize a modified place conditioning test to assess how CB exposure in adolescence affects COC's dual positive and negative effects. In this test rats are conditioned to associate a unique environment with the effects of COC present either immediately or 15 min after IV COC injection. Characteristically following place conditioning rats exhibit a preference for the environment paired with COC's immediate/positive effects (a CPP), and an aversion for the environment paired with COC's delayed/negative effects (a CPA). Therefore, we assessed the impact of CB exposure in adolescence on adult COC reward and aversion. Adolescent male rats were treated with one of three doses of the synthetic CB WIN 55,212-2 (WIN; 0.5mg/kg, 2mg/kg, 5mg/kg) or its vehicle (VEH) once per day for eight days (PND35-42). Following treatment rats were left in their home cages until they reached adulthood at which point they underwent place conditioning for either the immediate/positive or delayed/negative effects of COC (PND77-86). Interestingly, while rats treated with VEH during adolescence developed the typical pattern

of COC CPP and CPA, exposure to WIN during adolescence dose-dependently resulted in the development of CPA for the immediate effects of COC (an outcome opposite to the canonical COC CPP), while not disrupting CPA for COC's delayed/negative effects. These data suggest that CB exposure in adolescence diminishes COC's rewarding effects in adulthood and, in fact, results in a predominantly negative experience within the first 5 min after administration (i.e. the length of each conditioning trial). It remains unclear how these alterations in COC reward translate to COC seeking in adulthood. In animal models, however, reduced COC reward is associated with increased COC intake, suggesting that these observed decrements in COC reward might contribute to increased COC administration.

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**315.14/J24. *Intra-bed nucleus of the stria terminalis (BNST) pituitary adenylate cyclase activating peptide (PACAP) infusion reinstates cocaine seeking in rats***

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The tendency of users to relapse severely hinders adequate treatment of addiction. Physical and psychological stressors often contribute to difficulties in maintaining behavior change, and may play a significant role in relapse. We have previously shown that the activation of pituitary adenylate cyclase activating peptide (PACAP) systems in the bed nucleus of the stria terminalis (BNST) mediate many consequences of chronic stressor exposure. Hence, chronic stress substantially increased BNST PACAP levels, intra-BNST PACAP infusions produced the behavioral and endocrine consequences of stressor exposure, and BNST PACAP antagonism blocked many of the consequences of chronic stress. In the present set of studies, we investigated the role of BNST PACAP in stress-induced reinstatement of cocaine seeking. All rats self-administered cocaine (3mg/ml; 0.5mg/kg/infusion, i.v.) for 1hr daily over 10 days followed by extinction training in which lever pressing no longer resulted in cocaine delivery. In the first experiment we showed that intra-BNST PACAP infusion (1 µg; 0.5 µl per side) could reinstate previously extinguished cocaine seeking behavior. In the second experiment we found that intra-BNST infusions of the PAC1/VPAC2 antagonist, PACAP 6-38 (1 µg; 0.5 µl per side) blocked reinstatement following stressor exposure (5 sec 2mA footshock). Overall, these data suggest that BNST PACAP systems mediate stress-induced reinstatement to drug seeking. Understanding the neuropharmacology of BNST PACAP in stress-induced reinstatement and the role of PACAP systems may lead to viable targets for relapse prevention.

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**315.15/J25. *Antagonism of dopamine D4 receptors in the lateral habenula reduces the anxiogenic response to cocaine in a runway model of drug self-administration***

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Human users of cocaine report that the initial “high”, or period of euphoria, that results from taking the drug is temporally replaced by an aversive “crash” that is characterized as a period of agitation, anxiety and anhedonia. Our laboratory has previously reported that self-administered cocaine in rats produces behavioral effects consistent with the human report - that is, an initial experience of reward followed by period of anxiety and anhedonia. While the positive motivating effects of cocaine have long been thought to require an intact mesolimbic dopamine (DA) system, the neural mechanisms that give rise to the negative effects of the drug remain less clearly defined. Recent literature points to the lateral habenula (LHb) as a site for the encoding of aversive or anxiogenic events and has also been shown to “gate” the activity of the DA reward system by inhibiting the activity of DA cells within the VTA. This “gating” is hypothesized to stem from a negative feedback loop originating in the VTA, and involving DA projections to the LHb and direct and indirect reciprocal connections back to the VTA. The current study investigated the putative modulatory effects of DA stimulation on the aversive/anxiogenic properties of cocaine as measured in a runway model of IV self-administration. Male rats were stereotaxically implanted with bilateral cannulae aimed at the LHb and then trained to run a straight alley for IV cocaine (1.0 mg/kg) delivered upon arrival in a goal-box. As we have previously reported, vehicle pretreated controls developed approach-avoidance conflict behaviors about goal-box entry that are reflective of the dual positive and negative effects of IV cocaine. The frequency of such behaviors was significantly diminished in animals pretreated with bilateral intra-LHb injection of the D4 receptor antagonist, L-745,870. These results suggest that DA neurons in the VTA may not only be involved in producing the positive-rewarding effects of cocaine, but also actively subduing the negative after-effects of the drug. This work was supported by NIDA grant DA-033370 awarded to AE.

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**315.16/J26. *Activation of serotonin 1B autoreceptors in the bed nucleus of the stria terminalis attenuates the negative/anxiogenic effects of cocaine***

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Consistent with the Opponent Process Theory of motivated behavior, cocaine administration produces dual and opposing affective states: an initial rewarding “high” followed by a dysphoric/anxiogenic “crash”. It therefore seems likely that the motivation to seek cocaine is dependent upon the organism’s assessment of the positive relative to the negative consequences of its use. While the neurobiology of the reinforcing aspects of cocaine has been well established, less is known about the systems responsible for the drug’s negative actions. In this context, the current study involved an assessment of serotonergic (5-HT) function, which is enhanced by cocaine administration and has been linked to the presence of anxiogenic and depressive states in human and animal studies. In particular, we investigated the role of 5-HT within the bed nucleus of the stria terminalis (BNST) - structure within the extended amygdala that is activated during periods of stress and during the negative affective state associated with the withdrawal from drugs of abuse. The present study tested the hypothesis that increased 5-HT release in the BNST contributes to the anxiogenic effects of cocaine. A runway self-administration

paradigm was employed in which animals were trained to traverse a straight alley in order to earn an infusion of IV cocaine (1.0mg/kg) delivered upon goal box entry. Testing consisted of 16 single daily trials. In this task, animals develop ambivalence about goal box entry (reflected by the development of approach/avoidance “retreat” behaviors) that we have shown to reflect the dual positive (rewarding) and negative (anxiogenic) associations that subjects form with the cocaine-paired goal-box. To assess the involvement of 5-HT signaling within the BNST on this conflict behavior, prior to each trial rats received bilateral intracranial injections of CP94,253 (0.0µg, 0.5µg, or 1.0µg/side in 0.5/µl), a potent and selective 5-HT<sub>1B</sub> agonist that inhibits local 5-HT release via activation of terminal autoreceptors. Results indicated that CP94,253 did not alter the positive incentive properties of cocaine (start latencies were unaffected) nor did it alter gross motor behavior (as revealed in subsequent locomotor activity testing). Treatments did, however, selectively attenuate the negative effects of cocaine, as indicated by a dose-dependent decrease in the frequency of approach-avoidance “retreat” behaviors. We therefore conclude that 5-HT signaling within the BNST likely contributes to the negative/anxiogenic effects of cocaine. This work supported by NIDA grant DA-033370 awarded to AE.

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315.18/J28. ***Fos and dopamine activation in reward pathways of rats selectively bred for enhanced drug self-administration***

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Background: Vulnerability to psychostimulants varies greatly among different individuals, with genetic factors contributing to these differences. In one comparative analysis of humans with substance abuse disorders, heritability was highest for cocaine relative to other forms of drug abuse. The LS and HS rat lines were developed by selective breeding for low and high levels of intravenous drug self-administration in our laboratory. For low-dose cocaine, HS rats self-administer approximately five-fold more injections than LS animals. Here, we explored cocaine-induced dopaminergic activation in brain reward circuitry of LS and HS animals. Methods: Conditioned-place preference (CPP) testing was performed in three-chamber shuttle boxes using an un-biased procedure. Rats were conditioned with low dose (daily intraperitoneal injections of 0.7, 1.3, 2.7, and 5.3 mg/kg) or high dose (daily injections of 2.0, 4.0, 8.0, and 16.0 mg/kg) cocaine under an ascending-dose protocol for CPP, with cocaine preference tested three days after conditioning. Subsequently, animals were euthanized and brains examined for c-fos, dopamine d1 (D1R) as well as D2 (D2R) receptor activation in nucleus accumbens core (NAc) and shell (NASh), caudate putamen (CPu), ventral tegmental area (VTA) and dentate gyrus (DG) by immunofluorescent-staining analysis. Results: Both low and high dose cocaine induced CPP in both strains. Preferences were significantly larger in HS animals, with no significant interaction between strain and cocaine dose. Relative to the LS strain, both cocaine doses produced greater c-fos expression and activation of D1R and D2R neurons in HS rats, for all five brain regions. Compared to high-dose cocaine, D2R activation was significantly elevated in HS rats that received low-dose cocaine, but not LS animals. A similar pattern of increased D1R activation for low-dose treated HS rats was observed in

NASh, CPU, and VTA only. Conclusion: HS rats exhibit greater cocaine-induced CPP, fos activation, and activation of D1R and D2R relative to LS animals in various brain regions including the NAc, NASh, CPU, VTA and DG. HS rats are more sensitive to drug-induced activation of the dopamine system by low-dose cocaine in a subset of brain reward regions. This mechanism may underlie their enhanced sensitivity to cocaine.

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315.19/J29. ***Extended cocaine-seeking produces a shift from goal-directed to habitual responding in rats***

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Cocaine addiction is often characterized by a rigid pattern of behaviors in which cocaine users continue seeking and taking drug despite negative consequences associated with its use. As such, full acquisition and relapse of drug-seeking behavior may be attributed to a shift away from goal-directed responding and shift towards the maladaptive formation of rigid and habit-like responses. This rigid nature of habitual responding is typically characterized by insensitivity to changes in outcome value, which can be developed with extended training. Rats given extended access to self-administered cocaine are considered to have transitioned from a recreational or limited drug access to chronic use patterns indicated by an escalation of intake and higher reinstated behaviors. Additionally, extended access protocols offer an extensive number of drug trials that provides a nice framework to study drug-seeking habits following transition from recreational to chronic drug use. The present study determined whether cocaine (primary reinforcer) and cocaine associated cues (secondary reinforcer) could be devalued in rats with different histories of cocaine self-administration. All rats received one-hour cocaine self-administration sessions for 14 days, followed by either 1 day of six-hour sessions (long-access) or 14 days of continued one-hour sessions (short-access). Following acquisition, rats received outcome devaluation before undergoing a 7 day period of abstinence. The paired group received cocaine (administered i.v. through a playback program based on the number of infusions received on the last day of self-administration) immediately followed by an aversive compound lithium chloride (LiCl; 0.6 M, 10 ml/kg, i.p.) before being placed into holding cage. The unpaired group received LiCl injections 6 hours prior to cocaine infusions. Rats were given two reinstatement tests, the first in which they were exposed to the cocaine-paired cues only and the second in which rats were exposed to cocaine itself via contingent lever response. Cocaine history did not have an impact on devaluation of cocaine-associated cues. However, only rats on a short-access cocaine schedule displayed devaluation to the reinforcing properties of cocaine, but rats trained on a long-access schedule did not. Taken together this pattern of results suggests that, in short access rats, devaluation is specific to the primary reinforcer and not associative stimuli such as cues. Importantly, rats that received extended training during self-administration displayed insensitivity to outcome devaluation of the primary reinforcer as well as all associative stimuli.

315.20/J30. ***Chronic stress exposure during early withdrawal from extended access cocaine self-administration facilitates incubation of cue-induced cocaine craving***

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major challenge for treating cocaine addiction is the propensity for abstinent users to relapse. Two important triggers for relapse are cues associated with prior drug use and stressful life events. Human studies indicate that exposure to chronic adverse life events is associated with increased relapse vulnerability, indicating a need for animal models that explore interactions between chronic stress and drug withdrawal. However, the majority of studies investigating stress-induced relapse vulnerability have examined the effects of acute stressors on the reinstatement of previously extinguished drug seeking behavior, a model which may not accurately depict the situation of addicts, who typically do not undergo extinction training and may relapse after a long drug-free period. To study the effect of chronic stress on withdrawal-dependent changes in relapse vulnerability, we used the incubation model of craving and relapse in which cue-induced drug seeking progressively intensifies (“incubates”) during withdrawal from extended-access cocaine self-administration. Food restriction or repeated restraint stress were used as chronic stressors. Rats self-administered cocaine under extended-access conditions (6 h/d for 10 d) that have been shown to produce incubation of craving. On the day after the last self-administration session [withdrawal day (WD) 1], rats received a test for cue-induced cocaine seeking, during which nose-pokes resulted in presentation of the light cue but not cocaine. Rats were then divided into 2 groups destined for either control or stress conditions. In the food restriction studies, rats underwent a 2 week period of mild, chronic food restriction stress starting on WD2 (body weight maintained at 90% of their baseline weight). Control rats had ad libitum access to food. On WD15, rats underwent a second seeking test. In the repeated restraint studies, rats underwent 7 daily restraint sessions (20 min) over a 9 day period from WD6 to WD14 and received a seeking test on WD15, a day after the last repeated restraint session. Controls were placed in a cage with bedding on the same schedule. As expected, we found that controls showed greater cue-induced cocaine seeking on WD15 compared to WD1 (i.e. incubation of craving). Interestingly, rats in both stress groups showed a more robust increase in seeking on WD15, indicating acceleration or facilitation of incubation. Separate studies showed that the enhanced cocaine seeking observed was due to chronic and not acute stress. These data indicate that chronic stress during early withdrawal facilitates incubation of cocaine craving, which is thought to contribute to enhanced relapse vulnerability.

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316.01/J31. ***Rats that experience footshock-induced abstinence from methamphetamine self-administration exhibit increased prodynorphin mRNA in the nucleus accumbens***

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Methamphetamine (METH) is an illicit psychostimulant that is abused worldwide. Despite the longterm problem of METH addiction, cellular and molecular mechanisms involved in the transition from occasional to habitual drug use remain to be elucidated. Towards this end, our laboratory has investigated the effects of METH self-administration (SA) on the expression of neuroplasticity-associated genes within the dorsal striatum. Here we examined the influence of footshock on METH SA. We also measured potential changes on gene expression in the nucleus accumbens (NAc) of rats self-administering METH. Male Sprague-Dawley rats were trained to self-administer METH (0.1 mg/kg/injection, i.v.) or saline during twenty-two (9-hr drug access) sessions. After that time, foot-shocks were administered in increasing intensity over a period of thirteen sessions. The rats were then tested for cue-induced drug craving at 1 and 2 days post-shock and euthanized 9 days after the second extinction test. We extracted RNA from the nucleus accumbens (NAc), made cDNA, and ran quantitative polymerase chain reaction (PCR). Foot-shock caused the separation into two distinct SA groups: shock-resistant (SR) rats that continued to press the lever for METH despite the negative consequence and the shock-sensitive (SS) rats that significantly reduce their lever pressing. Our PCR results reveal that the SS group had a significantly increased expression of prodynorphin, but not of proenkephalin, mRNA levels, relative to the rats in the control and the SR groups. The increase expression of this neuropeptide in the SS group suggests that the two groups of rats that respond differently to footshocks also differ in their expression of this kappa receptor agonist. Our data suggest that this model of METH SA with adverse consequences may provide greater insight into the mechanisms of relapse to METH in clinical situations. Acknowledgement: This work is supported by the Department of Health and Human Services/ National Institutes of Health/ National Institute on Drug Abuse/ Intramural Research Program

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316.04/J34. ***Acid-sensing ion channels control locomotor and rewarding effects of amphetamine***

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Drug addiction is a persistent mental illness and there is no effective therapy for patients. The precise mechanisms underlying addictive responses have not been completely deciphered. New evidence has been shown that ion channels in the brain reward circuits are believed to play a vital role in drug addiction. Acid-sensing ion channels (ASICs) are highly expressed in brain with ASIC1a and ASIC2 channels being the predominant subtypes. These channels are enriched at synaptic sites and are central for the regulation of normal synaptic transmission. Moreover, increasing evidence is linking ASICs to the pathogenesis of various neurological and neuropsychiatric disorders. We and others have shown that ASICs are involved in cocaine addiction. Here, we hypothesized that amphetamine, a psychostimulant similar to cocaine, may also impact the function of ASICs. Following IACUC approval, adult wild-type (WT) C57BL/6J, ASIC1 and ASIC2 knock-out (KO) mice were placed in individual test chambers to allow accommodation to novel environment for 60 minutes. They then received a single intraperitoneal (i.p) injection of amphetamine at 3.0 mg/kg, and their locomotor activities were recorded for 150 minutes.

The experiment was repeated daily for total of days. After 2-week withdrawal period, the mice were brought back to the behavioral chamber followed by a final challenge i.p injection of amphetamine at 1.5 mg/kg. Locomotor activity to this challenge dose was measured for 15 min. Acute amphetamine injection induced typical dose-dependent increase in locomotor activities in WT, ASIC1 and ASIC2 KO mice. However, the increase in locomotor activities were attenuated in ASIC1 and ASIC2 KO mice as compared to WT mice. Both WT, ASIC1 and ASIC2 KO mice showed sensitization to amphetamine. However, ASIC1 KO mice showed more, while ASIC2 KO mice showed less behavioral sensitization to amphetamine. Our data provides new understanding of the complex genetic and molecular mechanisms of ASICs in response to amphetamine exposure.

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316.05/J35. ***Individual differences in methamphetamine self-administration model methamphetamine-addicted phenotype and are associated with cellular alterations in dentate gyrus of hippocampus***

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The hippocampus is important for the relapse stage of addiction, and maladaptive patterns of methamphetamine intake result in altered levels of neurogenesis in the dentate gyrus during withdrawal. Individual differences in methamphetamine intake during extended-access self-administration were used to model the methamphetamine-addicted phenotype. These individual differences allowed us to test whether specific patterns of methamphetamine-intake differentially altered proliferation and survival of neural stem cells, and granule cell neuron activation and structural plasticity in the dentate gyrus of the hippocampus. Male outbred Wistar rats were trained to self-administer methamphetamine hr/day for 1 sessions (FR schedule, 0.05mg/kg per IV infusion). Rats with higher levels of methamphetamine intake (high responders, n = 15) exhibited escalating patterns of drug intake across sessions, whereas the rats with lower levels of methamphetamine intake (low responders, n = 13) maintained a stable pattern of intake without escalation. High responders exhibited vertical and rightward shift in the self-administration dose-response function and higher breakpoints on a progressive ratio schedule compared to low responders, indicating a preferred higher intake level and higher motivation. BrdU was injected during withdrawal and after three weeks of withdrawal, high responders demonstrated greater latency to extinguish drug-seeking behavior, greater drug-context-induced reinstatement and greater cue-induced reinstatement, indicating higher propensity for drug relapse compared with low responders. Brain tissue was processed for Golgi-Cox staining, and hippocampal sections were processed for Ki-67 (cell proliferation), BrdU (17-day-old surviving cells), AC3 (apoptosis), and cFos (neuronal activation) immunohistochemistry. Stereological analysis of cell numbers in the dentate gyrus resulted in an increase in expression of Ki-67, BrdU, and cFos in high responders, and an increase in AC3 in low responders compared to controls. 3D Sholl analysis of

preexisting dentate gyrus granule cell neurons demonstrated no differences in dendritic complexity. The changes in behavior and associated cellular effects were not due to differential metabolism or bioavailability of methamphetamine since hippocampal methamphetamine levels were identical 45 min after methamphetamine challenge in low and high responders. These findings suggest that methamphetamine addiction is related specifically to differential alterations in granule cell neurogenesis, and these alterations may be able to modulate methamphetamine-seeking behavior.

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316.06/J36. ***Investigating the role of nucleus accumbens core astrocytes in reinstated methamphetamine seeking***

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Astroglial cells play an important role in the regulation of synaptic plasticity in the nucleus accumbens core (NAcore) through the regulated release and uptake of glutamate. Numerous studies show that exposure to various drugs of abuse causes reductions in the expression and or function of key components of the glial glutamate release (cystine-glutamate exchanger, system Xc-) and uptake (glial glutamate transporter, GLT-1) machinery. Importantly, these drug-induced neuroadaptations have been linked to relapse vulnerability. Consistent with other stimulants, methamphetamine (meth) self-administration followed by extinction training reduces extracellular glutamate in the NAcore leading to a potentiation of corticoaccumbal synapses, which underlies the enhanced glutamate release following exposure to drug-paired cues responsible for the initiation of drug seeking. We hypothesized that meth self-administration followed by extinction would also decrease expression and/or function of GLT-1. Strikingly, we did not observe any reduction of GLT-1 expression or glutamate uptake. Further, ceftriaxone, a  $\beta$ -lactam antibiotic, which has been shown to restore Xc- and GLT-1 expression, normalize glutamate uptake, and inhibit reinstated cocaine seeking, had no effect on cued meth seeking. However, restoration of NAcore glutamate tone via chronic N-acetylcysteine treatment during extinction inhibited cued-induced meth reinstatement. Additionally, activation of Gq-coupled designer receptors exclusively activated by designer drugs (DREADDs) in NAcore astrocytes prior to cue-induced reinstatement stimulated glial glutamate release and inhibited cued meth seeking, while having no impact on cued sucrose seeking. In summary, our data demonstrate that meth self-administration and extinction training did not impact accumbens glutamate uptake and that ceftriaxone treatment did not inhibit cued meth reinstatement. In contrast, restoration of extrasynaptic glutamate tone with N-acetylcysteine or activation of astroglial Gq-DREADD receptors reduced cue-induced meth seeking. Combined, our data indicate that similar to other drugs of abuse, potentiated synaptic glutamate release following exposure to meth-paired cues is responsible for the initiation of drug seeking and despite the lack of alterations in glutamate uptake, cued reinstatement of meth seeking is inhibited by the normalization of extrasynaptic glutamate tone provided by enhanced glial glutamate release.

**316.07/J37. *Neuron-specific modulation of dendritic spine dynamics in nucleus accumbens by amphetamine-paired contextual stimuli***

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Repeated exposure to amphetamine leads to both associative conditioning and nonassociative sensitization. Here we assessed the contribution of immunohistochemically identified cFos+ and FosB+ medium spiny neurons (MSNs) in the nucleus accumbens (NAcc). Compared to saline exposed rats, animals exposed to amphetamine IP or in the ventral tegmental area (VTA) showed the expected sensitized locomotor response when challenged with amphetamine weeks later. Both exposure routes also increased FosB levels in medial aspects of the NAcc, suggesting a role for FosB+ MSNs in this site in the accrual and maintenance of sensitization. Further characterization of these FosB+ neurons with single-cell injections of the neuronal tracer Dil, however, revealed no differences between saline and amphetamine exposed rats in dendritic spine density or in spine tip diameter, indicating that these neurons do not undergo changes in dendritic spine morphology that accompany the expression of nonassociative sensitization. As drugs are necessarily administered in the presence of a large number of environmental stimuli, conditions favoring the formation of drug-stimulus associations, additional experiments determined how NAcc MSNs contribute to the expression of associative conditioning. In these experiments, a discriminative learning paradigm was used to expose rats to IP or VTA amphetamine either Paired or Unpaired with an open field. In addition, Paired rats received saline and Unpaired rats amphetamine in the home cage. Control rats received saline in both environments. As expected, Paired rats administered amphetamine IP showed a conditioned locomotor response and an increase in the number of cFos+ neurons in medial NAcc when subsequently challenged with saline in the open field. Paired rats previously exposed to VTA amphetamine showed no evidence for conditioned locomotion and no evidence for an increase in the number of cFos+ neurons. An increase in FosB+ neurons was observed in both Paired and Unpaired rats, again consistent with a role for these neurons in the accrual of sensitization but not in the expression of conditioning. Further characterization of the activated cFos+ MSNs revealed that IP amphetamine exposed Paired rats, compared to rats in the other groups, showed an increase in the density of dendritic spines and spine tips as well as in the frequency of medium-sized spine tip diameters. These findings suggest a role for cFos+ MSNs in the medial NAcc and specifically for rapid changes in the morphology of their dendritic spines in the expression of conditioned responses evoked by amphetamine-paired stimuli.

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**316.08/J38. *Therapeutic potential of perirhinal cortex DREADDs on methamphetamine-induced deficits in novelty recognition and relapse***

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Background: Long-term methamphetamine (meth) abuse has been linked to certain cognitive impairments in humans. Similarly, in rats, chronic meth self-administration leads to deficits in novel object recognition memory, which relies upon the perirhinal cortex. One central question is: how do these cognitive deficits impact reinstated meth seeking? Here, our purpose was to determine whether synthetic activation of the perirhinal cortex with DREADDs could repair meth-induced novelty recognition deficits and to investigate the impact of these cognitive enhancing effects on relapse using a novel cue-choice reinstatement test. Methods: We used a viral-mediated gene transfer approach to infect perirhinal neurons with designer receptors exclusively activated by a designer drug (DREADDs of the hM3Dq variant) in order to activate neurons. All rats were infused with AAV2-hSyn-HA-hM3Dq-IRES-mCitrine vector (UNC Vector Core) bilaterally into the perirhinal cortex prior to meth self-administration, therefore allowing at least 4 weeks for DREADD expression to peak. Rats self-administered meth (0.02 mg/infusion, i.v.) along an FR1 schedule of reinforcement. After 7 daily 1-h sessions, rats were switched to 6-h daily access sessions for 14 days, and then underwent drug abstinence. Rats were tested for object recognition on abstinence day 7 and 8 or 14 and 15, and tested for novel cue-choice relapse on abstinence day 7 or 14. Neuronal activation was achieved by administering the designer drug clozapine-N-oxide (CNO, 10 mg/kg, i.p.); control rats received vehicle. Results: Chronic meth self-administration resulted in an escalation of meth intake over time and pronounced object recognition deficits. CNO administered immediately after object familiarization effectively restored object recognition in meth rats 90 min later. Twenty-four hours later, however, the therapeutic effects were no longer evident. In contrast, CNO had no impact on novel cue-choice reinstatement when administered 30 min prior to testing. Discussion: The data suggest that synthetic activation of the perirhinal cortex is capable of restoring novelty recognition in chronic meth-exposed rats. However, these therapeutic effects did not extend to reduced relapse in a model incorporating choice between a novel cue and a meth cue. Further research is needed to determine whether CNO dose or pharmacokinetics can account for the lack of effect on relapse. Nonetheless, restoring cognitive function in meth addicts using a DREADD approach is a translationally attractive means to help sustain abstinence.

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316.09/J39. ***Amphetamine affects reward-related behavior but not reward-evoked dopamine signals***

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Dopamine (DA) transients in the nucleus accumbens (NAc) are brief increases in DA that are critical for reward-related learning. They are elicited by unpredicted rewards and learned cues that predict rewards but not predicted rewards. Thus, learning correlates with a “transfer” of NAc DA transients from rewards to their predictive cues. The DA transfer deficit theory of attention deficit hyperactivity disorder (ADHD) proposes that symptoms are caused by insufficient “transfer” and that psychostimulant medications compensate for this by enhancing cue-evoked DA transients. At therapeutic doses, amphetamine (AMPH) is the most efficacious treatment for ADHD; however, at larger doses AMPH is rewarding and can cause addiction. Rewarding doses of AMPH enhance DA transients, an effect thought

to contribute to addiction via over-learning of drug-predictive cues. The effect of therapeutic doses of AMPH on DA transients has not been investigated; therefore, it is unclear if AMPH-induced enhancement of DA transients is specifically a mechanism of reward learning or also a therapeutic mechanism. We employed a Pavlovian autoshaping paradigm to examine AMPH dose effects in rats. This paradigm uses insertion of a lever and illumination of a light on one side of the chamber as CS+; insertion of a lever and illumination of a light on the opposite side is used as a CS-. Some rats, goal-trackers, approach the food-trough when the cue is present while other rats, sign-trackers, approach the lever. Prior to the second of ten conditioning sessions, rats received an i.p. injection of saline or AMPH (0.25 or 1.36mg/kg d-amphetamine hemi-sulfate). Rats treated with the high dose increased sign-tracking and tended to require more sessions to learn to respond to the CS+ and not the CS-. We also combined fast-scan cyclic voltammetry with an unpredicted food reward paradigm to determine if alterations in reward-evoked DA transients might contribute to these behavioral outcomes. A carbon-fiber microelectrode was lowered into the NAc until reward-evoked DA transients could be reliably recorded. 30 food pellets were delivered on a variable schedule both before and after administration of 0.25 or 1.36mg/kg AMPH. Additionally, electrically evoked phasic-like DA signals were recorded before and after the behavioral task. Only rats treated with the high dose exhibited increased electrically evoked signals. Surprisingly, the high dose had no significant effect on reward-evoked transients yet it tended to cause the rats to cease consuming the rewards. Taken together, these experiments suggest rewarding doses of AMPH alter learning by a mechanism that does not alter reward-evoked DA transients.

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316.10/J40. ***Inhibition of AKT phosphorylation in the rat ventral tegmental area prevents intermittent social defeat stress-induced weight gain deficits and the expression of amphetamine cross-sensitization***

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Intermittent social defeat stress is an unpredictable stressor that produces cross-sensitization to amphetamine and corresponding upregulation of mu-opioid receptors (MORs) in the ventral tegmental area (VTA). A population of VTA gamma-aminobutyric acid (GABA) neurons express MORs, which inhibit GABA release onto local dopamine neurons, and their expression is necessary for social stress-induced cross-sensitization to amphetamine. We previously showed that social stress increases the labeling of phosphorylated AKT (pAKT) preferentially in VTA GABA neurons, and that this effect is dependent on VTA MOR expression. We hypothesize that intra-VTA inhibition of pAKT during social defeat stress will prevent stress-induced amphetamine cross-sensitization and weight gain deficits. Adult male Sprague Dawley rats received bilateral cannulas directed at the VTA. Social defeat stress consisted of exposure to both threat of defeat and a brief physical defeat by an aggressive Long-Evans rat. Either saline vehicle or NVP-BE2235 (10  $\mu$ M in 1  $\mu$ l per side), a dual inhibitor of phosphoinositide 3-

kinase/mTOR signaling used to inhibit AKT phosphorylation, were infused 1 hr prior to each episode of social defeat stress or control handling, which occurred 4 times in 10 days. Intra-VTA inhibition of pAKT significantly blocked the development of long-term weight gain deficits observed in vehicle-treated stressed rats. Ten days after the last episode of defeat, all rats received an amphetamine challenge (1.0 mg/kg, i.p.). Intra-VTA inhibition of pAKT during stress did not alter the development of stress-induced amphetamine cross-sensitization, however when inhibitor was infused one week later prior to a second amphetamine challenge, it completely blocked the expression of cross-sensitization, as locomotor activity of stressed rats did not differ from handled animals after VTA pAKT inhibition. Taken together, these data implicate MOR-induced pAKT in the metabolic and behavioral effects of stress, suggesting that VTA pAKT may mediate stress-induced weight gain deficits and vulnerability to psychostimulants. Furthermore, that intra-VTA inhibition of pAKT blocked the expression, rather than the induction, of amphetamine cross-sensitization suggests that pAKT inhibition may provide a novel therapeutic approach for the treatment of substance abuse.

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316.13/J43. ***Differential rearing alters amphetamine self-administration: Role of mGluR2/3 activation***

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Previous research has demonstrated that rats reared in an enriched environment self-administer less amphetamine at low unit doses than rats reared in isolation. We have recently demonstrated that differential rearing influences the function of post-synaptic metabotropic glutamate receptors (mGluRs) that contribute to the maintenance of glutamate homeostasis. The current study sought to determine if differential rearing also influences the function of presynaptic mGluRs critical for glutamate homeostasis, as research suggests that maintaining homeostatic glutamatergic function may be a protector against drug abuse. Male rats arrived to the lab at 21 days of age and were assigned to enriched (EC), isolated (IC), or standard (SC) conditions. EC rats were handled by experimenters and lived with cohorts and novel objects. IC rats lived with neither cohorts nor novel objects. SC rats lived in pairs in shoebox cages to provide a lab standard for comparison. At 52 days of age, rats were trained to lever press for 20% sucrose on a fixed-ratio schedule (FR-1). Rats were then implanted with indwelling jugular catheters. Following surgery recovery, rats self-administered intravenous amphetamine (0.1 mg/kg/infusion) on a FR-1 schedule during 6 min daily sessions. After reaching stable responding, rats were injected with three doses (0, 0.3, and 1.0 mg/kg, i.p.) of the mGluR2/3 agonist, LY-379268 (LY), 30 min prior to self-administration sessions. Following FR-1 testing with all three doses, rats were tested with the same doses of LY on a progressive-ratio (PR) schedule following the same design as the FR-1 phase. Results revealed that LY generally decreased amphetamine self-administration under both FR-1 and PR schedules of reinforcement. Differential rearing influenced the attenuation of FR-1 self-administration. Specifically, EC rats given both 0.3 and 1.0 mg/kg LY earned significantly fewer amphetamine infusions than IC rats given the same doses. Furthermore, time course analyses revealed that LY led to a greater suppression of FR-1 and PR amphetamine self-administration in EC rats

compared to IC rats. 1.0 mg/kg LY suppressed early-session FR-1 amphetamine self-administration in EC rats compared to IC rats, with no time course differences observed during vehicle FR-1 self-administration sessions between any of the environmental groups. These findings suggest the mGluR2/3 receptor may play a role in altering amphetamine self-administration among differentially reared rats, and differential rearing alters the function of pre- and post-synaptic mGluRs that maintain glutamate homeostasis.

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**316.16/J46. *The effect of reactive oxygen species scavengers in methamphetamine-taking behaviors and dopamine release in the nucleus accumbens***

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Methamphetamine (METH), a powerful and commonly used psychostimulant, has been shown to induce reactive oxygen species (ROS) formation, leading to oxidative stress, by dopamine (DA) auto-oxidation at DAergic terminals. Recently, we and others have implicated ROS in the development of behavioral sensitization following repeated contingent and non-contingent administration of psychostimulants such as cocaine. In this study, we evaluated the involvement of ROS in METH self-administration behavior and acute METH enhancement of DA release in the nucleus accumbens (NAc) using fast scan cyclic voltammetry (FSCV) in vivo. To evaluate the effect of ROS scavengers, rats received N-tert-butyl- $\alpha$ -phenylnitron (PBN, a nonspecific ROS scavenger; 50 or 75 mg/kg, IP) or 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL, a SOD mimetic; 50 or 100 mg/kg, IP) 10 minutes prior to beginning of METH self-administration (0.05 mg/kg/infusion) and IV injection of acute METH (0.1 mg/kg). Systemic administration of PBN or TEMPOL significantly decreased METH self-administration without affecting food intake. Using 8-OHG immunohistochemistry, increased oxidative stress was found in the NAc of rats self-administering METH compared to sham controls. Acute administration of TEMPOL (100 mg/kg, IP) had no significant effect on the enhancement of DA release produced by acute METH. However, in preliminary studies, repeated administration of TEMPOL (25 mg/kg, 4 days, IP) decreased DA release produced by acute METH. Taken together, these findings indicate that enhancement of ROS production contributes to the reinforcing effect of METH.

**316.17/J47. *Acute methamphetamine induces hydrogen peroxide formation in dopamine terminals of the nucleus accumbens***

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Methamphetamine (METH) is a powerful psychostimulant known to both reverse the dopamine (DA) transporter (DAT) and inhibit the monoamine vesicular transporter (VMAT-2). Ultimately, METH facilitates DA release by causing reverse transport of DA through the DAT while simultaneously interfering with VMAT-2 pumping of DA into vesicles. Dopamine is subject to auto-oxidation and enzymatic degradation via MAO, resulting in formation of reactive oxygen species (ROS), specifically hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The aim of this study was to evaluate the role of ROS in the effects of acute METH on phasic and basal DA release in the NAc. Utilizing a fluorescent dye sensitive to peroxide formation and fast-scan cyclic voltammetry (FSCV), superfusion of METH (1-100  $\mu$ M) induced H<sub>2</sub>O<sub>2</sub> production in the NAc. Using FSCV and the antioxidants glutathione (GSH) and 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL), we evaluated the role of ROS in acute METH enhancement of both phasic (modeled by electrical stimulation) and basal DA release. Superfusion of METH (0.1-100  $\mu$ M) dramatically increased phasic DA release in the NAc slice preparation (330% at 100  $\mu$ M METH). However, the enhancement was transient, for within 15 min of continuous superfusion of METH DA release returned back to baseline, suggesting desensitization. This effect was difficult to wash out - over an hour later similar challenge dose of METH was unable to raise the signal more than 5%. Glutathione (100  $\mu$ M) was unable to attenuate the transient increase in phasic DA release, but was able to prevent METH's enhancement of basal DA release. Similarly, TEMPOL, a SOD mimetic, had no effect on phasic release, but attenuated basal release. Inhibiting DAT with GBR 12909 was unable to impact the effects of METH on phasic DA release, but inhibiting VMAT-2 with tetrabenazine reduced the effects of METH on phasic DA release. Since VMAT-2 appears to be playing a primary mechanistic role in basal release and a small role in phasic release, protein mechanistic studies are underway to show interactions between GSH and cysteine residues on VMAT-2.

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316.18/J48. ***Selenium deficiency alters dopamine transmission and response to methamphetamine in the mouse nucleus accumbens***

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Selenium (Se) is an antioxidant trace element that is important for normal brain function. Se is incorporated into selenoproteins, a family of proteins with multiple functions that include protection from oxidative stress. Methamphetamine (METH) increases dopamine (DA) signaling by inhibiting DA reuptake, resulting in increased oxidative stress from oxidized DA, and eventual degeneration of DAergic terminals. Previous studies have indicated that Se protects against METH-mediated neurotoxicity, potentially through the antioxidant actions of the glutathione peroxidase (GPx) selenoenzymes. Conversely, Se-deficiency potentiates METH toxicity. To investigate the mechanisms of how dietary Se deficiency alters METH toxicity, we investigated DA concentrations and reuptake kinetics in the nucleus accumbens (NAc). We used fast-scan cyclic voltammetry (FSCV) to measure changes in extracellular DA

in NAc brain slices following evoked release and changes following METH application. Se-deficiency impaired initial DA reuptake kinetics compared with slices from mice raised on a normal Se diet. This indicates reduced function of the dopamine active transporter (DAT). Se-deficiency did not alter the METH-induced increase in peak extracellular DA concentration. However, Se-deficiency did attenuate METH-induced impairments of DA reuptake kinetics. We additionally measured protein changes in the brains of Se-deficient mice compared to mice on a normal diet. Western blots demonstrated that Se-deficiency decreased levels of DAT and GPx compared to controls. These results suggest that Se-deficiency results in less availability of DA reuptake machinery, promoting DA toxicity and impairing responses to METH.

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317.01/K6. ***Se differences in novel object recognition after a binge methamphetamine treatment***

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Methamphetamine is a well-established neurotoxin that selectively damages dopaminergic cells, causing cell death and destruction of dopamine terminals. Behavioral evidence also indicates an effect on dopamine-dependent activities, as exemplified by the novel object recognition task. However, few studies have examined the effects of methamphetamine on females. This study sought to test the sex differences in memory impairment using the novel-object recognition task. A four-dose methamphetamine “binge” dosage paradigm (4 x 5mg/kg, 2 hours apart) was used to compare memory performance between males, intact females, and ovariectomized females. Drug-treated males and ovariectomized females, but not intact females, exhibited memory impairments compared to saline animals. No differences in total exploration time were observed based on sex or drug condition. Methamphetamine toxicity-induced behavioral deficits therefore appear to differentially impact individuals based on sex.

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317.02/K7. ***Methamphetamine-induced aberrant neurogenesis: Protection by exercise***

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While no effective therapy is available for the treatment of methamphetamine (METH) induced neurotoxicity, behavioral interventions, including aerobic exercise, are being used to improve depressive symptoms and substance abuse outcomes. The present study focuses on the effect of exercise on METH-induced neurotoxicity in the hippocampal dentate gyrus (DG) in the context of the blood-brain barrier (BBB) pathology. METH or saline (vehicle) was administered three times per day for 5 days with an escalating dose regimen at 4 h intervals. One set of mice was sacrificed 7 day post last injection of

METH and the remaining mice were divided into two major groups: a) the exercise group and b) the sedentary group. After chronic METH administration, the expression of tight junction (TJ) proteins was decreased in the hippocampus. Importantly, BBB permeability was significantly increased and remained elevated even 2 days after the withdrawal of METH. Moreover, neuronal differentiation was significantly decreased in METH-exposed hippocampal DG, suggesting impaired neurogenesis. Most importantly, voluntary exercise protected against this effect, enhanced the protein expression of occludin, and inhibited induction of inflammatory cytokines. These results suggest that exercise can attenuate METH-induced neurotoxicity by protecting against the BBB disruption and related microenvironmental changes in the hippocampus.

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317.06/K11. ***Neurotoxic consequences of serial exposure to alcohol and methamphetamine***

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Methamphetamine (Meth) and alcohol have an established comorbid relationship in drug abuse. Despite the widespread co-abuse of these drugs, little is known about the consequences arising from their serial exposure. Glutamate signaling is altered by alcohol and Meth administered independently, and glutamate-induced excitotoxicity mediates Meth neurotoxicity. Therefore, we tested the hypothesis that the serial exposure to alcohol and Meth will alter glutamatergic transmission and result in greater brain monoamine depletions than either drug alone. Male Sprague Dawley rats were exposed to a one week chronic binge of ethanol (6g/kg/day) via oral gavage followed by one day of binge Meth administration (10mg/kg x injections). One week after treatment, the monoamine neurotransmitter content of the prefrontal cortex, striatum, and hippocampus was measured. Ethanol alone did not result in any monoamine depletions, while rats treated with Meth alone showed a 50% depletion of dopamine in striatum, as well as a 25% depletion of serotonin in striatum, hippocampus, and prefrontal cortex. Importantly, ethanol significantly enhanced the neurotoxicity observed after Meth alone in that rats treated with both drugs showed 90% dopamine depletions within the striatum and 75% serotonin depletions in the striatum, hippocampus and prefrontal cortex. Other markers of monoamine terminals, such as dopamine transporter (DAT), serotonin transporter (SERT) and tyrosine hydroxylase (TH) immunoreactivities in the striatum were also measured one week after treatment. Western blot analysis showed that DAT, SERT and TH immunoreactivities were decreased 80% in the striatum of ethanol + Meth rats, compared to 40% decreases in rats exposed to Meth alone and no changes after ethanol alone. This enhanced effect after serial exposure to ethanol and Meth suggests a potential synergism between the drugs. To determine a role for glutamate in mediating monoamine depletions, the excitatory amino acid transporter (EAAT1) was examined. EAAT1 immunoreactivity in the prefrontal cortex was decreased by 24% at one day after a week of ethanol alone. These results suggest a potential role for diminished glutamate uptake in mediating a vulnerability to excitotoxicity produced by ethanol, which could potentially be enhanced by Meth. Future studies will examine the role of excitotoxicity in mediating neurotoxicity after alcohol and Meth.

317.10/K15. ***MDMA reduces markers for GABAergic neurons in the hippocampus and increases seizure susceptibility: Role of glutamate mediated excitotoxicity***

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MDMA is a unique psychostimulant that continues to be a popular drug of abuse. It is well documented that MDMA produces persistent reductions in markers of 5-HT axon terminals in rodents, as well as humans. To date there has been little recognition of potential MDMA neurotoxicity to neuronal populations beyond 5-HT axon terminals in brain regions, such as the hippocampus, in which damage may account for the neurologic/cognitive effects associated with repeated exposure to MDMA. In the present study, we examined the hypothesis that MDMA produces glutamate-dependent damage to GABAergic neurons, as assessed from GAD67-positive neurons in the hippocampus, which results in an increase in seizure susceptibility. Repeated exposure to MDMA (3x10mg/kg, ip) resulted in a marked reduction in the number of GAD67 positive cells in the dentate gyrus, as well as in the CA1 and CA3 regions. Repeated administration of MDMA also resulted in an increased susceptibility to kainic acid-induced seizures that persisted for at least 30 days following MDMA treatment. Kainic acid (9 mg/kg, sc) produced seizures in approximately 20% of control animals, whereas approximately 85% of MDMA-treated animals exhibited kainic acid-induced seizures. The MDMA-induced increase in seizure susceptibility was not evident in rats treated with either MK-801 (a NMDA antagonist) or ceftriaxone (an inducer of GLT-1). In further support for a role of glutamate-mediated excitotoxicity in the MDMA-induced loss of hippocampal GABA neurons and increase in seizure vulnerability, is the finding that repeated treatment with MDMA results in an increased extracellular concentration of glutamate in the hippocampus that is also prevented in rats treated previously with ceftriaxone.

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317.11/K16. ***MDMA decreases paired-pulse depression and afterdischarge threshold in the dentate gyrus: Roles of 5HT2a and EP1 receptor activation***

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MDMA is a widely abused psychostimulant which causes the release of serotonin through its actions on the serotonin transporter. Recent findings in our lab demonstrated that MDMA causes an increase in extracellular glutamate concentrations within the dentate gyrus, which was dependent upon local activation of 5HT2A receptors. These increases in glutamate were also dependent upon local activation of EP1 receptors, suggesting a role for PGE2 signaling in mediating these glutamate increases. Here we report that local administration of MDMA (100  $\mu$ M) causes a significant increase in PGE2 concentrations within the dentate gyrus (246%, p=.016) of rats. PGE2 increases were inhibited when MDL100907, a 5HT2a receptor antagonist, was administered with MDMA. Previously, we reported significant

decrease in parvalbumin (PV) interneurons in the dentate gyrus following MDMA exposure, which could be prevented by inhibition of either 5HT<sub>2a</sub> or EP1 receptors during MDMA exposure. Given the previously demonstrated decreases in PV interneurons following MDMA exposure, we investigated whether MDMA alters inhibition within the dentate gyrus. Perforant path evoked field potentials in the dentate gyrus of MDMA treated rats exhibited a reduced paired-pulse depression at 40, 50 and 65 ms interstimulus intervals (41.5, 47.2, 36.4%,  $p=.001$ ,  $.003$ ,  $.006$  respectively), 10 days following MDMA exposure (7.5 mg/kg x 4, ip). Decreases in paired-pulse depression were prevented by treatment during MDMA exposure with either MDL100907 or SC-51089, an inhibitor of EP receptors. Further experiments revealed decrease in the stimulus amplitude needed to drive perforant path induced afterdischarges in the dentate gyrus of MDMA treated rats (19.4%,  $p=.001$ ). Reductions in afterdischarge threshold were prevented when rats were treated during MDMA exposure with either SC-51089 or MDL100907. These findings suggest that MDMA causes a decrease in inhibition within the dentate gyrus, which may disrupt the excitatory/inhibitory balance. Furthermore, these findings suggest that MDMA-induced reductions in PV interneurons are responsible for decreases in inhibition. Further studies are needed to characterize the changes in GABAergic inhibition within the hippocampus of MDMA treated animals and whether these changes mediate known deficits in hippocampal function caused by MDMA.

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317.12/K17. ***Clinically-relevant pharmacological strategies that reverse MDMA-induced brain hyperthermia potentiated by social interaction***

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MDMA-induced hyperthermia is highly variable, unpredictable, and greatly potentiated by the social and environmental conditions of recreational drug use. Current strategies to treat pathological MDMA-induced hyperthermia in humans are palliative and marginally effective, and there are no specific pharmacological treatments to counteract this potentially life-threatening condition. Here, we injected rats with a moderate non-toxic dose of MDMA under conditions modeling recreational drug use (i.e., social interaction) that led to the dramatic enhancement of MDMA-induced brain hyperthermia. We tested the effectiveness of mixed adrenoceptor blockers carvedilol and labetalol, and the atypical antipsychotic clozapine in reversing MDMA-induced hyperthermia. To mimic the clinical situation of drug intoxication, we injected the treatment drugs after MDMA had already caused robust hyperthermia ( $>2.5^{\circ}\text{C}$ ). Brain temperature was our primary focus, but we also simultaneously recorded temperatures from the deep temporal muscle and skin, allowing us to determine the basic physiological mechanisms of the treatment drug action. Carvedilol induced skin vasodilation and was modestly effective in attenuating MDMA-induced brain and body hyperthermia, whereas labetalol was ineffective. In contrast, clozapine induced a marked and immediate reversal of MDMA-induced hyperthermia via inhibition of brain metabolic activation and blockade of centrally-mediated vasoconstriction. Our findings suggest that clozapine, and related centrally acting drugs, can be highly

effective for reversing MDMA-induced brain and body hyperthermia in emergency clinical situations, with possible life-saving results. Supported by NIDA-IRP.

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317.13/K18. ***Sex-differences in rodent methamphetamine self-administration***

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Methamphetamine (METH) is a widely abused psychostimulant that can cause persistent alterations in brain neurochemistry. METH is abused in both females and males. Research has shown that women exhibit higher co-morbid neuropsychiatric disorders and prefer METH to other drugs compared to their male counterparts. However, little research has been conducted to understand these sex-differences. The purpose of the present study was to investigate possible sex-differences in the behavioral and neurochemical effects of METH self-administration. Male and female rats were subjected to 7 days of self-administration (8 hours/day) of either METH or saline and were sacrificed one hour after the last self-administration session. METH-induced changes in hippocampal brain derived neurotrophic factor (BDNF) and expression of the striatal dopamine transporter (DAT) were assessed. Similar METH self-administration occurred between the sexes; however, METH-induced hyperthermia was significantly greater in females. METH self-administration decreased striatal DAT immunoreactivity in both females and males. METH-induced increases in hippocampal BDNF immunoreactivity occurred in males but not in females. In conclusion, there are similar drug-taking behaviors between the sexes, but sex-differences exist in the neurochemical consequences of METH self-administration. These findings may have clinical implications to sex-differences observed in human METH users.

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317.17/K22. ***Extended-access to cocaine has distinct behavioral and molecular profile from yoked- and limited-access to cocaine***

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Cocaine addiction is a chronic disorder that involves escalation of intake over time. Approximately 10% of the US population has used cocaine by the age of 18, yet, only about 1% of US citizens meet the criteria for cocaine addiction. Both humans and rodents will self-administer escalating doses of cocaine when the drug is readily available. However, when cocaine access is restricted, escalation of intake is not apparent in rodents. Here, we investigated escalation of cocaine intake and active lever responding in rats as a function of control over intake. Rats were implanted with a permanent jugular catheter and then allowed to lever press to self-administer (FR1, 20s time-out with a 20s light cue paired with each infusion) saline vehicle (0.1 ml/infusion) cocaine (0.25 mg/infusion) under 4 conditions: limited-access (1

h/ day) to saline, limited-access to cocaine, extended-access (6 h/day) to cocaine condition, and limited-access yoked-access (1h/day h/day, respectively) to cocaine. Based on the first 1 min and first hour of daily access, we observed rapid escalation of cocaine intake in both the extended-access and limited-access yoked conditions ( $p < 0.05$ ). We also observed delayed escalation of cocaine intake in the limited-access condition within the first 10 min of self-administration ( $p < 0.05$ ), but not within the first 1 h of self-administration. Interestingly, there was an immediate escalation of active lever responding in the limited + yoked-access condition during the first 1 minutes of daily self-administration which preceded the escalation of responding observed in the extended-access condition.

similar pattern of escalation for active lever responding was observed during the first 1 of daily self-administration for all cocaine conditions. However, relative to the other cocaine conditions, the limited-access yoked group exhibited markedly less efficient self-administration (i.e. more non-reinforced relative to reinforced lever responses) during both the first 10 min and 1 h of daily self-administration ( $p < 0.05$ ). . Additionally, post-mortem quantification of homer2 (a gene implicated in cocaine cued learning) mRNA expression within the dmPFC indicated elevation only in the extended-access conditions ( $p < 0.05$ ). Together, these findings indicate that either contingent or non-contingent “excessive” cocaine exposure supports escalation but has distinct effects on the temporal patterning of operant responsiveness as well as molecular correlates of escalation.

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### 317.18/K23. *Chronic cocaine disrupts angiogenesis and cerebral blood flow in the mouse brain*

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Cocaine-induced stroke is among the most serious medical complications associated with its abuse. However, the extent to which chronic cocaine-induced reductions in cerebral blood flow (CBF) and micro-ischemia affects cerebral blood vessels has not been investigated, in part, because of the lack of tools with high spatiotemporal resolution and sensitivity that can simultaneously measure cerebral blood flow velocity (CBFv) quantitatively and morphology with single vascular resolution over large fields of view. Recently, we developed novel optical imaging techniques that permitted the quantitative CBF imaging of cerebrovascular networks along with the imaging of cortical vascular angiography simultaneously. Our study showed that CBF was decreased in response to cocaine and the micro-ischemia was observed in the cortex of the animal after repeated cocaine administration. Here we extend the study to investigate the effects of chronic cocaine on CBF and in the morphology of cerebral blood vessels. To monitor the neurovascular changes from chronic cocaine exposure in mice, a cranial window was implanted on the cortex of each individual mouse. Two groups of animals were used; a control group (saline, ~0.1cc/10g/day, i.p. 35 days) and a cocaine group (30mg/kg/day, i.p. 35 days). Each animal was periodically scanned to image CBFv and angiography of the cortical vascular network simultaneously till the end of treatment. Our repeated imaging of the cortex through the implanted cranial window showed vasoconstrictive effects of cocaine on the brain vessels with chronic cocaine treatment. Vessel constriction induced decreases in CBFv as compared to their baseline (prior to cocaine

treatment). The diameters of vessels were decreased ~ 25-35% after 3 days of cocaine exposure compared to baseline, whereas CBFv in these vessels was decreased ~ 40-50%. Interestingly, angiogenesis surrounding the constricted vessels was observed by 12-14 days of cocaine treatment. Blood flow gradually developed in these new growing vessels and 7-8 days later blood flow to the local area had increased 20-30% and was maximal at the end of 3 days of our experiment. These results indicate that the angiogenesis is a response to repair the local micro-ischemia of brain induced by cocaine. Although the angiogenesis intends to improve blood perfusion into the ischemic brain area, the limited CBFv within these vessels makes them difficult to fully compensate for cocaine-induced cerebrovascular dysfunction.

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317.19/K24. ***Amphetamine and morphine may produce aspects of acute withdrawal by initially affecting common pathway***

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When separate groups of rats are administered 2.0 mg/kg amphetamine or 5.0 mg/kg morphine near light onset of a 12-12 hour light-dark cycle, activity is reduced 18 to 24 hours later. This longer-term hypoactivity may be an indicator of an acute withdrawal. If a dopamine D1 receptor antagonist is given shortly after amphetamine or morphine, the reduction in activity appears to be blocked. The similar time courses with which amphetamine and morphine produce hypoactivity, and the similar effects of D1 antagonist on the occurrence of the hypoactivity, suggest that the drugs produce it by initially activating common circuit. This study sought additional evidence that amphetamine and morphine produce longer-term hypoactivity via a common pathway. Though all rats show longer-term hypoactivity following amphetamine or morphine, the magnitude differs across subjects. If amphetamine and morphine produce longer-term hypoactivity by initially activating a common circuit, then the magnitude of hypoactivity following amphetamine and morphine might be expected to be correlated, the hypothesis assessed in the present study. Adult male Wistar rats were individually housed in open field arenas (43 cm X 4 cm X 3 cm high), equipped with grids of infrared emitters and detectors. Beam interrupts were used to monitor distance moved per unit time, our measure of activity. The animals were in a 12-12 hour light-dark cycle and had free access to water and food (Purina rodent chow). After animals had habituated to the arenas and entrained to the light-dark cycle, they were tested at intervals of five days. On the first day of a test (Day 1), near light onset, animals received a saline administration. Two days later (Day 3), near light onset, they received 2.0 mg/kg amphetamine or 5.0 mg/kg morphine. Station maintenance occurred at the time of treatment. Activity was monitored for 2 hours following Day 1 and Day 3 treatments. Activity following drugs was compared to activity following saline. During the first six tests, amphetamine was administered, and during the final tests, morphine was administered. The magnitude of hypoactivity that different animals showed following amphetamine and morphine appeared to be qualitatively similar.

317.20/K25. ***Disruption of the hubs of the connectome in cocaine addiction. A multimodal approach***

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Brain networks or 'connectomes' are organized around highly connected processing 'hubs', which are particularly valuable for efficient information processing. Disruptions of these crucial information processing hubs have been linked to impairments in multiple neuropsychiatric disorders, including dementia, schizophrenia, and post-traumatic stress disorder. A recent meta-analytic study has demonstrated that structural microlesions in hu regions predict disruptions of these regions in resting-state functional connectivity. Using a novel multimodal neuroimaging approach, we aim to investigate both if the functional resting-state connectome in cocaine addiction shows disruptions specifically in brain processing hubs, and if these disruptions are predicted by structural microlesions. We acquired structural, diffusion-weighted and functional resting-state data in cocaine users (n=30) and healthy controls (n=33), matched on race, gender and intelligence. Per individual, a whole-brain functional connectome was derived from the resting-state data following standard procedures: parcellating the data according to an anatomical template, calculating functional connectivity between each pair of regions, and thresholding each connectome to contain only the strongest connections. Diffusion-weighted data were parcellated using the same template, and the number of fiber tracts connecting each pair of regions was computed. Brain connectivity (or degree) was defined as the number of connections between each region with all other brain regions. Structural data was processed using the same template, computing changes in gray matter volume by voxel-based morphometry. Functional and structural connectomes of cocaine addicts were contrasted with controls', controlling for ag ( $p < 0.05$ , uncorrected). Ongoing work is using logistic regression to determine associations between structural and functional connectome abnormalities. Cocaine users showed disruptions of resting-state connectivity in processing hubs, such as the anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (dlPFC), hippocampus, putamen and caudate that have previously been implicated across brain disorders. The ACC, dlPFC, and hippocampus also showed reduction in structural brain connectivity. We expect these disruptions to correlate with abnormalities in gray matter volume. Results support functional and structural abnormalities in brain network hubs within the cognitive control network in cocaine addiction. Evidence of their correlation could advance a multimodal systems-level account of the neural circuitry involved in compulsive behavior.

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317.21/K26. ***Characterizing atherosclerosis in asymptomatic cocaine addicted individuals***

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Cocaine use is involved in 40% of emergency department visits, where positive toxicology for illicit drugs have been associated with stroke, coronary artery disease and myocardial infarction, resulting in severe impairments or sudden mortality, even in absence of prior vascular disease symptoms. Indeed, cocaine, powerful vasoconstrictor and sodium channel blocker, decreases basal anti-inflammatory markers (Interleukin 10) and increases pro-inflammatory cytokines (Tumor necrosis factor alpha), contributing to progressive vascular inflammation (atherosclerosis). The carotid arteries supply blood to brain regions that are implicated in the higher-order cognitive impairments documented in individuals with cocaine use disorder (iCUD). Hence, structural and/or functional damage in the carotid arteries may impact cognitive and behavioral functioning even before substantial arterial narrowing results in clinical symptoms. We hypothesized that iCUD have significant vascular inflammation, which is modulated by history of drug use. Therefore, using Positron Emission Tomography/Magnetic Resonance (PET/MR), we imaged the internal carotid arteries to assess atherosclerosis in 10 healthy iCUD aged 43 to 58 with cocaine lifetime use of  $22.6 \pm 7.3$  years and without a history of neurological or cerebrovascular disease (CVD). We compared results to findings in a sample at risk for CVD, aged  $64.6 \pm 7.8$ . Amount of inflammation, measured with PET with 18F-fluorodeoxyglucose (18F-FDG), was calculated by the maximum arterial wall (target) to background (blood) ratio (TBR). To measure enlargement of wall area and thickness of the vessel, we used MR with 3-Dimensional dark-blood sequence. Results show that 78% of iCUD had inflamed plaque in arteries [TBRmax. (mean, SE) right (1.89, .12) left (1.7, .11); notably, TBR 1.6 is indicative of inflamed plaque]. Furthermore, in one sample t-tests using the comparison group's mean values, iCUD had thicker wall (mm; 1.63, .03 versus 1.27, .04,  $t(8)=8.84$ ,  $p=.00$ ) and larger wall area (mm<sup>2</sup>; 38.45, 1.48 versus 32.28, 1.43,  $t(8)=3.34$ ,  $p=.01$ ) indicating the presence of more plaque in the carotid than the much older comparison sample at risk for CVD. These PET/MR findings correlated significantly with cocaine use indices (lifetime use, craving, and addiction severity) and with nicotine and alcohol lifetime use where the more severe the drug use, the greater the carotid abnormalities ( $.53 \leq r \leq .85$ ,  $p < .01$ ). These preliminary results show markers of carotid disease in CVD-asymptomatic iCUD, which may exacerbate cognitive and behavioral impairments, of paramount clinical significance for combating silent disease progression.

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318.01/K27. ***Endocannabinoid system alterations in an animal model of autism spectrum disorders***

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Alterations of the endocannabinoid system (ECS) are involved in the pathophysiology of neuropsychiatric disorders including autism spectrum disorders (ASDs). The interaction between genes and environmental factors including immune system dysregulation are associated with ASDs. The ECS consists of the cannabinoid receptors (CB1Rs and CB2Rs), endocannabinoids (eCBs), and the synthesizing and degradation enzymes of eCBs. ECS are involved in embryo neurodevelopment and growth and is a key regulator of the immune system via CB2Rs which are expressed on macrophages, microglial cells and neurons. We used the BTBR T+tf/J mice that have been shown to exhibit autism-like behavioral phenotypes to 1). Determine brain expression of CB2Rs throughout neurodevelopment in BTBR T+tf/J and C57BL/6J mice and also to measure the levels of eCBs, anandamide (AEA) and 2-arachidonolyl glycerol (2-AG) in frontal cortex, cerebellum and the rest of the brain by LC-MS using isotopic dilution method. 2). Evaluate the neurochemical and molecular basis of cannabinoid-induced behavioral effects and 3). Determine impact of SERT, DAT, MOR, and DAT-CI gene knock out on CBR-induced behaviors in motor function and emotionality tests. We report that CB2Rs are present and essential during neurodevelopment and its enhanced brain expression in the adult BTBR mice might be associated with the differential cannabinoid-induced behavioral effects when compared to the C57BL/6J mice. But [3H] CP55,940 binding to CB1Rs did not differ between BTBR and C57BL/6J mice in the amygdala and parietal cortex. CB2R agonist, JWH133 and ACEA- CB1R agonist reduced motor activity in both BTBR and C57BL/6J mice. ACEA induced aversive behavior in both the BTBR and C57BL/6J mice while CB1R antagonist-AM251 reduced aversive behavior in both BTBR and C57BL/6J strains. In the transgenic mice, the effect of JWH133 was genotype and gender dependent in the motor function and emotionality tests. SERT ko mice were more active in the wheel running activity (WRA) and this was enhanced by JWH133 in the male but not female SERT ko mice. Similar reductions in WRA were recorded for the male and female DAT, DAT-CI and MOR ko mice. In MOR and DAT-CI ko mice, JWH133 induced aversions but reduced aversions in SERT and DAT ko male mice in the two chamber black and white box. AEA but not 2-AG levels in the BTBR mice were reduced in the brain areas analyzed. The data indicate that dysfunction in the ECS may in part contribute to ASDs and other neuropsychiatric disorders. Further studies are required to determine the contribution of the different elements of the ECS involvement in the etiology of ASDs.

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318.02/K28. ***Mechanisms and signaling downstream the cannabinoid receptor 1/beta-arrestin***

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protein-coupled receptors (GPCRs) are transmembrane proteins that transduce external stimulus into intracellular effector pathways. When activated by ligands, they can elicit multiple signaling cascades that are mediated by G proteins or by multifunctional scaffold proteins known as beta-arrestins. While G protein pathways are well defined, the mechanisms and pathways downstream from beta-arrestin signaling are less understood. Here, we sought to investigate the mechanisms and signaling cascades

downstream from the cannabinoid 1 receptor (CB1R)/beta-arrestin. First, we tested the hypothesis that the duration of the interaction between CB1R/beta-arrestins during the endocytic process, can control beta-arrestin signaling. We characterized ligand-induced endocytosis of the CB1R in real time by total internal reflection (TIRF) microscopy at the single endocytic level in human embryonic kidney (HEK)293 cells and hippocampal neuronal cultures. Cells were transfected with the CB1R tagged with super-ecliptic phluorin (SEP). Endocytosis was initiated by bath application of different ligands and the endocytic dwell time, which is the time receptors are clustered with beta-arrestins at the endocytic pits before endocytosis, for each ligand was analyzed. We identified ligand-specific endocytic dwell times. The endogenous eicosanoid, 2-arachidonoylglycerol (2-AG), elicited prolonged dwell times (>120 seconds) and strong beta-arrestin signaling, whereas the synthetic agonist WIN 55,212-2 (WIN) elicited short ones (<120 seconds) and no beta-arrestin signaling. Furthermore, chemical inhibition of endocytosis significantly increased beta-arrestin signaling. In addition, by using antibody arrays and siRNA technology, we identified specific signaling pathways controlled by beta-arrestins. Our results indentify the signaling network downstream from CB1R/beta-arrestins and propose molecular mechanism controlling this type of signaling. Furthermore, we propose modulation of receptor trafficking as novel approach to control beta-arrestin mediated signaling.

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318.03/K29. ***Elucidating cannabinoid biology in zebrafish***

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Although the number of annual cannabis users exceeds 100,000,000 globally and an estimated 9% of these individuals will suffer from dependency, a dearth of knowledge exists about the potential consequences on public health. However, the psychoactive constituents of cannabis are known to signal through the endocannabinoid (eCB) system, and to disrupt features of vertebrate physiology and behavior. While studies have revealed that the eCB anandamide (AEA) regulates stress response system (SRS) activity, little is known about the pathological consequences of disrupted AEA signaling. Our central hypothesis is that disruptions in the AEA signaling system have pathological consequences on vertebrate behavior and physiology, including dysregulation of the SRS. Herein, we use a preclinical zebrafish model to clarify the ramifications of disturbances in the AEA signaling system. Using qRT-PCR and in situ hybridization we show that the genes encoding enzymes that synthesize (abhd4, gde1, napepld), enzymes that degrade (faah, faah2a, faah2b), and receptors that bind (cnr1, cnr2, gpr55-like) AEA are expressed throughout development. We show that disruptions of this system via exogenous cannabinoid administration results in altered behavior and physiology, including increased secretion of glucocorticoids in our stress response reporter line. We are developing a zebrafish AEA signaling mutant library using transcription activator-like effector nucleases (TALENs). Currently, we are identifying our first mutant lines and will share the preliminary results of behavioral assays using our first mutants. Collectively, these results establish zebrafish as a viable model for studying AEA signaling, and lay a

foundation for informing a better understanding of the toxicological and therapeutic potential of the eCB system.

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318.04/K30. ***Cannabinoid cb1 and cb2 receptors mediate the classical tetrad effects of delta9-tetrahydrocannabinol in mice***

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In animals, cannabinoid agonists such as delta9-THC produce characteristic combination of tetrad symptoms - hypothermia, analgesia, hypoactivity, and catalepsy. However, the receptor mechanisms underlying these actions are incompletely understood. When cannabinoid CB1 and CB2 receptors were first cloned in 1990s, CB1 receptor was found in the brain and periphery, while CB2 receptor was found only in periphery. Therefore, it is believed that brain CB1, not CB2, receptor mediates the action produced by cannabis. This view has been challenged by recent finding that functional CB2 receptor is expressed in the brain and in midbrain dopamine neurons. This new finding inspired us to re-examine whether brain CB2 receptor is also involved in the action produced by cannabis such as delta9-THC. To address this issue, we first compared the behavioral response to delta9-THC under the same experimental conditions between wild-type (WT) and CB1 receptor-knockout (CB1-KO) or CB2 receptor-knockout mice (CB2-KO). We found that delta9-THC, at 1 or 3 mg/kg (i.p.), produced dose-dependent analgesia (as assessed by hot-plate test), hypothermia, catalepsy and rotarod performance impairment in WT mice, but not in CB1-KO mice. Surprisingly, deletion of CB2 receptor in CB2-KO mice also blunted delta9-THC-induced analgesia and catalepsy. We then observed the effects of the selective CB1 receptor agonist (ACEA) or CB2 receptor agonist (JWH133) in WT mice. We found that, systemic administration of ACEA (1-10 mg/kg, i.p.) or JWH133 (1-10 mg/kg, i.p.) alone failed to produce significant effects in the above measurements, while co-administration of ACEA and JWH133 produced significant analgesia, hypoactivity and catalepsy. These findings suggest that 1) brain CB1 receptor plays a predominant role in mediating delta9-THC-induced tetrad effects; 2) brain CB2 receptor also play an important role in mediating delta9-THC-induced analgesia and catalepsy; and 3) co-activation of brain CB1 and CB2 receptors are required in mediating the behavioral effects produced by cannabinoid ligands. (Supported by NIDA IRP)

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318.05/K31. ***Differential tolerance produced by daily administration of Δ9-THC and JWH-018 in rhesus monkeys***

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The Cannabis derivative  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) and the synthetic cannabinoid naphthalen-1-yl-(1-pentylindol-3-yl) methanone (JWH-018) are both CB1 receptor agonists; however, unlike  $\Delta^9$ -THC, JWH-018 has been linked to adverse effects such as seizures and hypertension. The disparity between adverse effects could be due to JWH-018 having higher CB1 receptor efficacy than  $\Delta^9$ -THC, evidenced most commonly by greater maximum stimulation of inhibitory G proteins. In a previous study, daily  $\Delta^9$ -THC treatment produced tolerance and cross-tolerance to JWH-018 in non-human primate model of subjective effects; however, tolerance to  $\Delta^9$ -THC was greater than cross-tolerance to JWH-018, consistent with a difference in CB1 receptor efficacy. What remains unclear is the extent to which daily JWH-018 administration alters sensitivity to JWH-018. This study tested the hypothesis that tolerance to  $\Delta^9$ -THC is greater than tolerance to an equally effective dose of JWH-018, as would be predicted for chronic treatment with a low versus a high efficacy agonist. Rhesus monkeys (*Macaca mulatta*) discriminated  $\Delta^9$ -THC (0.1 mg/kg i.v.) from vehicle under an FR5 schedule of lever pressing to avoid a noxious stimulus. Both  $\Delta^9$ -THC and JWH-018 produced dose-dependent increases in  $\Delta^9$ -THC lever responding to 100%; the respective ED50 values were 0.026 and 0.0084 mg/kg, a difference of 3-fold. The time courses of discriminative stimulus effects following subcutaneous administration were compared:  $\Delta^9$ -THC (1 mg/kg) produced 80%  $\Delta^9$ -THC lever responding for 8-12 h, whereas the duration of action of JWH-018 (0.32 mg/kg) was 4-8 h. According to these time courses,  $\Delta^9$ -THC (1 mg/kg s.c.) was administered once daily and JWH-018 (0.32 mg/kg s.c.) was administered twice-daily 6 h apart.  $\Delta^9$ -THC treatment produced 3.8-fold loss of sensitivity after three days and a 16-fold loss of sensitivity after 7 days. JWH-018 treatment produced 2.1-fold loss of sensitivity after three days and a 4.7-fold loss of sensitivity after 7 days. Consistent with the hypothesis, tolerance to the low efficacy CB1 receptor agonist  $\Delta^9$ -THC was greater than tolerance to the high efficacy CB1 receptor agonist JWH-018. The differential development of tolerance among CB1 receptor agonists as a function of efficacy might underlie the greater incidence of adverse effects following use of synthetic cannabinoids as compared with Cannabis.

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318.07/K33. ***Morphine and ethanol reward, tolerance, and dependence in mice expressing a desensitization-resistant form of the cannabinoid receptor 1 (CB1)***

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The focus of this study was to investigate whether enhanced endocannabinoid system (ECS) signaling modulates reward, tolerance, and dependence for morphine and ethanol. Preference and consumption of ethanol is attenuated in CB1 knockout mice using a two-bottle choice voluntary drinking paradigm. Studies also demonstrate involvement of the CB1 receptor in the modulation of reward, dependence and tolerance to morphine. We produced S426A/S430A mutant mice expressing desensitization-resistant form of CB1 to study the importance of desensitization for tolerance to cannabinoid agonists. During the characterization of these novel mutant mice, we found that they also display an exaggerated

and prolonged acute response to  $\Delta^9$ -THC and endogenous endocannabinoids. Given the important role of CB1 in modulating the effects of opioid and ethanol reward and dependence, we decided to use the S426A/S430A mutant mice as a novel model to examine the effect of enhanced ECS signaling on these processes. Ethanol intake was measured using a 24-hour, continuous-access voluntary drinking paradigm. We found that S426A/S430A mice consumed significantly more 6 and 9% ethanol. Tolerance to ethanol-induced ataxia was examined using the rotarod, while tolerance to morphine-induced antinociception was assessed using the hotplate and tail-flick tests. Despite differences in ethanol intake, S426A/S430A and wild-type mice exhibit equivalent ethanol and morphine tolerance. Likewise, S426A/S430A also develop normal and robust CPP for morphine and cocaine. Morphine dependence was determined by counting naloxone-precipitated withdrawal symptoms in mice implanted with 75mg morphine pellets. Both genotypes displayed similar severity of morphine dependence; however, we found that S426A/S430A mutants recovered from precipitated withdrawal more quickly than wild-type littermates. These findings suggest that CB1 modulation of reward for, tolerance to, and dependence on ethanol and morphine is not heavily impacted by elevated ECS sensitivity due to disruption of CB1 desensitization. Although the S426A/S430A mice may indeed possess enhanced sensitivity to exogenously administered cannabinoids, our data suggests that they have limited utility for drug addiction research.

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318.09/K35. ***Tolerance to the antinociceptive effects of  $\Delta^9$ -THC in the formalin model of inflammatory pain***

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The use of cannabinoids to manage pain is of interest given the rise of opiate abuse among the general population.  $\Delta^9$ -THC produces potent antinociceptive effects but, like opiates, is subject to tolerance. The objective of this study was to examine the complete time-course of  $\Delta^9$ -THC tolerance in a model of inflammatory pain. The effect of  $\Delta^9$ -THC was assessed in male wild-type C57BL/6 mice subjected to inflammatory pain using formalin (10  $\mu$ l at 2.5 % intraplantar). Experimental groups were subjected to the formalin test after receiving chronic daily injections of  $\Delta^9$ -THC (6 mg/kg), or vehicle, for periods of time starting from zero consecutively up to eight days. We also examined the effect of JNK inhibitor SP600125 on  $\Delta^9$ -THC. Control groups injected with saline for 1, 4, and 8 days were examined. Mice tested after one day of exposure showed the greatest level of antinociception while mice treated for eight days showed almost no antinociception. Preliminary data suggest that co-administration of SP (3 mg/kg) with  $\Delta^9$ -THC prolongs the onset of full tolerance from 2 to 4 days. In phase I (acute pain), tolerance develops gradually across eight days, with the largest jump between two and three days of chronic administration. While also gradual across eight days, in phase II of the formalin test, the largest increase seems to occur between three and four days of chronic administration. These results are consistent with the findings in other models such as acute pain showing onset of tolerance by three days

of daily administration. Further studies are needed to investigate the longer retained efficacy of  $\Delta^9$ -THC appearing in inflammatory pain relative to acute pain in the tail-flick test. The findings of this study reinforce the validity of the formalin model as a pathologically relevant tool to assess cannabinoid tolerance in mice models. Also, it provides a detailed day-by-day map of the progression of  $\Delta^9$ -THC tolerance. Preliminary results also suggest that JNK signaling is involved in this observed tolerance. Acknowledgements: Funded by NIH grants DA036385 (DJM), DA037355 (DJM), and funded by Texas Tech University Health Sciences Center School of Medicine grant 121035(JG).

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### 318.10/K36. *Development of a novel rodent model of THC self-administration*

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Cannabis is the most frequently used illicit drug worldwide, but preclinical research on its effects has been hampered by lack of an animal model since rats will not maintain self-administration of isolated  $\Delta^9$ -tetrahydrocannabinol (THC), the drug's main psychoactive component. THC on its own may produce unpleasant side effects including increased anxiety, but cannabis contains over 60 cannabinoids and more than 400 additional chemicals. Cannabidiol (CBD), a non-psychoactive cannabinoid, may counter some of the aversive properties of THC by producing anxiolytic and anti-psychotic effects. Thus in the present investigation, one strategy that was employed to promote drug taking in male Sprague-Dawley rats was by combining CBD with THC in a ratio of 10:1, a proportion previously demonstrated to neutralize a THC conditioned place aversion. Moreover, we used a Volcano vaporizer to pre-expose rats to THC:CBD vapor (10 mg THC vapor per pad) for 3 days prior to initiating intravenous THC:CBD self-administration (4 g/kg/0.05 ml infusion) as it has previously been demonstrated that THC pre-exposure facilitates formation of a THC conditioned place preference rather than aversion. We determined that our THC vapor pre-exposure provided a physiologically relevant dose of THC as we were able to measure a decrease in core body temperature after exposure. During self-administration, clear lever discrimination was observed with greater than 2-fold preference for the drug-associated lever. The rats sustained low levels of responding with an average of 10 infusions per 2-hr session although there was high inter-individual variability. We generated a dose response curve in (1.27, 4, and 12.64 g/kg) and found that highest responding was observed at 4 g/kg/infusion. Importantly, we also demonstrated both cue-induced and THC-primed (1 mg/kg, ip) reinstatement in animals extinguished from THC:CBD. This affords the opportunity to initiate a reverse-translational investigation of n-acetylcysteine (NAC) to test its effects on reinstatement as this drug has recently demonstrated efficacy in clinical studies of marijuana dependence. Additional studies will examine biomarkers of altered glutamatergic synaptic plasticity in the nucleus accumbens after THC self-administration. In summary, we have established a rodent model of THC self-administration allowing us to evaluate THC-dependent brain changes relevant to addiction and relapse.

318.12/K38. ***Modulation of CB1 cannabinoid receptor signaling and adaptation by D2 dopamine receptors***

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Gi/o-coupled CB1 receptors (CB1R) in striatum produce motor suppression and catalepsy and regulate motivation. Repeated CB1 agonist administration produces tolerance that is associated with CB1R desensitization and downregulation in the CNS, however CB1Rs in striatal circuits are somewhat resistant to these adaptations. CB1Rs co-localize with D2 receptors (D2R) on striatopallidal medium spiny neurons, and interaction between these receptors affects their activity and expression. CB1R and D2R can heteromerize, which switches their G-protein coupling from Gi/o to Gs. We examined D2R-CB1R interactions in G-protein activation using [<sup>35</sup>S]GTPγS binding in wild-type (WT) or D2R knockout (KO) mice and CHO cells stably expressing CB1R (CB1-CHO) or CB1 and D2R (CB1/D2-CHO). Maximal CB1R mediated G-protein activation was reduced in both dorsal and ventral striatum of D2R KO compared to WT mice. In dorsal striatum of WT but not D2R KO mice, incubation with both the CB1 agonist WIN55,212-2 (WIN) and D2 agonist quinolorane (Quin) produced less G-protein activation than WIN alone. Despite reduced CB1R mediated G-protein activation in D2R KO mice, adenylyl cyclase (AC) inhibition by WIN was enhanced in dorsal and unchanged in ventral striatum of D2R KO mice, suggesting that D2R might switch a subset of CB1Rs from Gi to Gs protein activation. Interestingly, maximal G-protein activation by D2R did not differ between CB1R KO and WT mice in dorsal or ventral striatum, but the Quin EC<sub>50</sub> value in dorsal striatum was greater in CB1R KO mice. Because D2R alters CB1R signaling, co-expression with D2R might contribute to resistance of striatal CB1R to agonist-induced adaptation. This was tested in model cell system. CB1-CHO and CB1/D2-CHO cells were treated for 24 hr with WIN or Quin alone or in combination. WIN treatment desensitized CB1R mediated G-protein activation in CB1-CHO cells without downregulating CB1Rs. Importantly, CB1R desensitization by WIN was reduced in CB1/D2-CHO cells, but co-treatment with WIN + Quin did not further reduce CB1R desensitization. Treatment of CB1-CHO cells with WIN + the A activator forskolin attenuated CB1R desensitization compared to WIN alone, suggesting that heteromer coupling to Gs could play a role in D2R mediated attenuation of CB1R desensitization. Quin treatment of CB1/D2-CHO cells desensitized D2R, but enhanced CB1R mediated G-protein activation, whereas WIN treatment did not affect G-protein activation by D2R. These results demonstrate asymmetric interactions between CB1R and D2R in G-protein signaling and receptor adaptation.

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318.14/K40. ***Cocaine self-administration up-regulates cannabinoid CB2 gene expression in mouse brain***

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We have recently reported that brain cannabinoid CB2 receptors (CB2Rs) regulate midbrain dopamine (DA) neuronal activity, nucleus accumbens DA release and intravenous cocaine self-administration in mice and rats (Xi et al., Nature Neuroscience, 2011; Zhang et al., PNAS, 2014). These findings appear to conflict with anatomic evidence that brain CB2 gene level is very low (~50-fold) compared to that in the periphery - e.g., spleen. We hypothesized that acute or chronic use of drugs of abuse may up-regulate brain CB2 receptor expression, and produce significant effects on brain function. To test this hypothesis, we first treated animals with a single injection or repeated injections of cocaine, or chronic cocaine self-administration, respectively, and then measured brain CB2 mRNA levels using quantitative real-time PCR (qRT-PCR) and in situ hybridization (ISH) assays. We found that: 1) chronic intravenous cocaine self-administration (1 mg/kg/infusion 5 infusions/day 2 weeks) significantly up-regulated (4-5 fold) CB2 mRNA expression in the prefrontal cortex (PFC) and striatum of mice compared to that observed in oral sucrose self-administration (control) mice or drug naïve mice. In contrast, single injection (10, 20, 30 mg/kg, i.p.) or repeated injections of cocaine (10 mg/kg, i.p. per day, for 7 days) (i.e. locomotor sensitization dose regimen) failed to alter brain CB2 mRNA expression as assessed by qRT-PCR; 2) ISH assays show similar findings - CB2 mRNA is significantly up-regulated in cortical and striatal neurons as well as VTA DA neurons. To determine the cell types of striatal GABAergic medium-spiny neurons (MSNs) expressing CB2Rs, we used D1- versus D2-eGFP transgenic mice and fluorescence activated cell sorting (FACS) techniques, and found that CB2 mRNA is mainly expressed in D2-MSNs (3~4-fold higher in D2-MSNs than in D1-MSNs) in normal subjects. Repeated cocaine administration (20 mg/kg, i.p. per day for 7 days) significantly up-regulated CB2 mRNA expression in D1-MSNs, but not in D2-MSNs. Taken together, these findings suggest that brain CB2 receptors are inducible and responsive to chronic cocaine abuse or repeated large doses of cocaine. Thus, these findings not only well explain the anti-addictive effects of CB2R agonists observed in cocaine self-administration mice, but also suggest that brain CB2Rs may constitute a new target in medication development for the treatment of drug addiction and possible other CNS disorders.

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319.01/K42. ***The novel dopamine D3 receptor antagonists CAB02-015 and BAK4-54 inhibit oxycodone self-administration and reinstatement of drug-seeking behavior in rats***

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The use of prescribed opioid analgesics such as oxycodone has been increased dramatically in recent years, which parallels the increases in prescription opioid abuse and drug-related deaths worldwide. Thus, understanding the rewarding properties of prescription opioids, and accordingly, developing effective pharmacotherapies for treatment of prescription opioid abuse has become a critical matter of public health. In the present study, we studied the rewarding and reinforcing properties of oxycodone in animal models of drug addiction, and the potential roles of the novel dopamine D3 receptor antagonists CAB02-015 or BAK4-54 in mediation of these effects in rats. We have found that animals rapidly acquired oxycodone self-administration in a dose range of 0.03 -0.24mg/kg/infusion, with a pattern that

is similar to those of heroin or cocaine. Pretreatments in drug trained rats with either CAB02-015 or BAK4-54 (0.4~10mg/kg, i.p.) inhibited oxycodone self-administration dose-dependently. In addition, repeated treatments with CAB02-015 (0.4, mg/kg, i.p. for days) during the extinction sessions (in which oxycodone was replaced by saline) dose-dependently accelerated the extinction of drug-seeking behavior (as indicated by the accelerated decline in responding on the lever previously associated with oxycodone in CAB02-015 versus vehicle treated groups) and inhibited reinstatement of oxycodone-seeking induced by oxycodone priming injection (1 mg/kg, i.p.). Taken together, these findings indicate that 1) oxycodone possesses similar addictive properties as heroin and cocaine; and 2) the novel D3 receptor antagonists CAB02-015 and BAK4-54 may have therapeutic potential for addiction treatment associated with prescription opioid use.

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319.02/L1. ***Effects of the non-opioid (+)-naltrexone and the peripherally active (+)-N-methylnaltrexone in rats self-administering the mu agonist remifentanil***

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Toll-like receptor 4 (TLR4) is a protein that detects lipopolysaccharide from gram-negative bacteria and is involved in activation of innate immune responses to foreign substances. TLR4 is expressed centrally and peripherally. It was recently reported that the non-opioid TLR4 antagonist, (+)-naloxone, decreases self-administration of remifentanil, short acting mu-opioid agonist, in rats, suggesting an involvement of TLR4 in the reinforcing effects of mu-opioids (J. Neurosci. 32: 11187). Using the CNS-active and peripherally acting TLR4 antagonists (+)-naltrexone and (+)-N-methylnaltrexone, respectively, the present study compared i) central vs peripheral components of this effect, and ii) the specificity of the effect by comparing effects on responding maintained under comparable schedules of remifentanil or cocaine injection, and food presentation. One group of rats was trained to self-administer remifentanil (0, 0.1-3.2 µg/kg/inj, IV) under fixed ratio 5-response schedule of reinforcement in sessions comprised of five 20-min components. The dose of remifentanil per-ratio completed was increased during successive components of the session to determine dose-effect curves. A second group was trained under a comparable schedule with cocaine injections (0, 0.03-1.0 mg/kg/inj), and third group with food reinforcement using 0 to 4 food pellets per ratio completed in the successive components. The TLR4 antagonist, (+)-naltrexone (3.2-56 mg/kg, sc), its peripherally acting analog, (+)-N-methylnaltrexone (32-100 mg/kg, sc), or vehicle were administered before selected daily sessions. Control response rates were an inverted U-shaped function of remifentanil or cocaine dose or amount of food, with maxima at 1.0 µg/kg/inj, 0.32 mg/kg/inj, or pellets/presentation, respectively. (+)-Naltrexone dose-dependently decreased the self-administration of remifentanil, cocaine, and rates of responding maintained by food presentation. In contrast, (+)-N-methylnaltrexone, at doses greater than active doses of (+)-naltrexone, did not significantly alter remifentanil self administration. The potency of (+)-naltrexone in decreasing

behavior was slightly greater with cocaine self administration than with responding maintained by either remifentanil or food reinforcement, for which there was no difference in potency. Overall the present study suggests that the (+)-naltrexone produced decreases in self-administration of remifentanil and cocaine are centrally mediated though generalized to behaviors maintained by various reinforcers. Supported by NIDA IRP (JLK, BRS, KCR) and DoD Contract PR110146 (LRW).

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319.07/L6. **Activation of calmodulin-dependent kinase II protein in the hippocampus of oxycodone self-administered adult C57Bl/6 mice**

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An epidemic of prescription opioid and heroin addiction is occurring in the United States, characterized by: 1) wide-spread abuse amongst all age groups, but with especially high numbers amongst adolescents 2) frequent morbidity and mortality due to overdose and 3) high rate of relapse and long-term persistence of drug addiction. The surge in abuse of opioids highlights the necessity for a greater understanding of this disease. An often underappreciated aspect of addictions is the role of learning and memory. For example, the hedonic aspects of the drug become associated with the act of drug taking. Also, neutral environments/stimuli that become associated with drug use can trigger drug craving, consolidation and retrieval of contextual memories. Indeed, it has been hypothesized that formation and persistence of contextual memories facilitates compulsive drug taking. The activation of calmodulin dependent kinase II (CamKII) in the hippocampus has been shown to be a critical molecular mechanism in memory formation. Furthermore, numerous drugs of abuse including the opioids, have been shown to require CamKII activity for maintenance of drug-taking. To examine the function of CamKII activation in the hippocampus during oxycodone-reward learning, we conducted a self-administration experiment. Adult C57Bl/6 male mice were implanted with an intravenous catheter and allowed to self-administer oxycodone or saline 4 hours/day for 14 days (0.25mg/nose poke). As a critical control, whenever an animal self-administered oxycodone, another animal passively received an infusion of oxycodone (oxycodone-yoked controls). Mice were then sacrificed and total hippocampal protein lysate was analyzed by Western blot. We found a statistically significant increase in the amount of phosphorylated-CamKII $\alpha$  protein in the hippocampus of oxycodone-self administered mice when compared to the oxycodone-yoked or saline controls, though no difference in phosphorylated CamKII $\beta$  or total CamKII protein of either isoform was detected. Furthermore, mice that received investigator-administered oxycodone for 14 days (3mg/kg/day) did not exhibit any change in total or phosphorylated CamKII protein, as determined by Western blot analysis. Taken together, these data strongly suggest that activation of CamKII $\alpha$  plays a critical role in opioid addiction-like settings specifically involving learning and memory, but not in the primary hedonic effects of the drug itself. Improved knowledge of hippocampal function in opioid addiction may help lead to the development of medications for either prevention and/or treatment of opioid addiction.

319.11/L10. ***Morphine modulates mouse hippocampal progenitor cell lineages via PKC $\epsilon$ -dependent ERK activation and TRBP phosphorylation***

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Morphine regulates adult neurogenesis by modulating miR-181a maturation and subsequent hippocampal neural progenitor cell (NPC) lineages. By using NPCs cultured from PKC $\epsilon$  or  $\beta$ -arrestin2 knockout mice and the MEK inhibitor U0126, we demonstrate that regulation of NPC differentiation via the miR-181a/Prox1/Notch1 pathway exhibits ligand-dependent selectivity. In NPCs, morphine and fentanyl activate ERK via the PKC $\epsilon$ - and  $\beta$ -arrestin-dependent pathways, respectively. After fentanyl exposure, the activated phospho-ERK translocates to the nucleus. Conversely, after morphine treatment phospho-ERK remains in the cytosol and is capable of phosphorylating TRBP, cofactor of Dicer. This augments Dicer activity and promotes the maturation of miR-181a. Furthermore, by using NPCs transfected with wild type TRBP, S $\Delta$ A and S $\Delta$ D TRBP mutants, we confirmed the crucial role of TRBP phosphorylation in Dicer activity, miR-181a maturation, and finally the morphine-induced astrocyte-preferential differentiation of NPCs. Thus, morphine modulates the lineage-specific differentiation of NPCs by PKC $\epsilon$ -dependent ERK activation with subsequent TRBP phosphorylation and miR-181a maturation.

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319.14/L13. ***Modeling prenatal and postnatal oxycodone exposure using self-administration***

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The abuse of opiates has emerged as a major public health issue. Of growing concern are the numbers of women abusing opiates during pregnancy, with a significant increase observed over the past decade. A large number of these women are using prescription opiates containing oxycodone. While neonatal abstinence syndrome is one consequence of prenatal opiate use, the long-term consequences associated with use have not been well-documented. Additionally, animal models of prenatal oxycodone exposure are lacking. To address this gap, we have established a prenatal oxycodone self-administration model in rats. The goal of these studies are twofold; 1) develop a model with high face validity that can be used to make informed predictions about potential long-term neurodevelopmental effects in offspring; and 2) utilize this model to better understand variations in female use patterns that may be a natural function of the reproductive state and environmental conditions. In the current set of studies cycling female Sprague-Dawley (200-225g) rats were fitted with indwelling jugular catheters. One week later females began daily 1h self-administration sessions in standard self-administration chambers (Med Associates) with one lever press resulting in one infusion of oxycodone (0.1 mg/kg/infusion). During this period, females were assessed daily for stage of estrous cycle. Following at least three weeks of daily

sessions, proestrus females were placed overnight with breeder males. Pregnancy was confirmed by the presence of sperm in the vaginal lavage. Self-administration sessions continued throughout pregnancy and during the postpartum period with doses adjusted based on increasing and decreasing bodyweight. On postnatal day 1 (PND1), half of the subjects had their pups removed while the other half remained with their pups. Additionally on PND1, brains were harvested from one male and one female offspring from each litter. Data indicate that levels of responding for oxycodone increase during pregnancy and even more so during the postpartum period. Preliminary findings suggest that the presence or absence of pups influences the level of responding. Gene expression studies are ongoing in PND1 brains as are studies examining the effects of prenatal oxycodone on maternal behavior and pup ultrasonic vocalizations. These findings represent the initial phase of model development in the area of prenatal opiate abuse.

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**319.16/L15. *Stress-induced activation of amygdalar corticotropin releasing factor neurons projecting to the locus coeruleus in morphine dependent rats***

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Chronic opiate exposure promotes the development of long-term adverse consequences including tolerance and physical dependence as a result of persistent alterations in brain neurons. The locus coeruleus-norepinephrine (LC-NE) system is implicated in arousal and cognition associated with the stress response. Previous electrophysiological studies have demonstrated that the LC-NE is sensitized to corticotropin-releasing factor (CRF) following chronic morphine exposure. While it is known that the LC receives CRF afferents from multiple brain regions including the central nucleus of the amygdala (CeA), the mechanism underlying enhanced sensitivity to stress following chronic opiate use remains unknown. In a first study, male Sprague-Dawley rats received morphine via subcutaneous delivery of pellets. Controls received placebo pellets. In situ hybridization labeling of CRF mRNA in the amygdala revealed a lack of statistical significance in expression levels across groups. To further characterize stress-induced neuronal activation of LC-projecting amygdalar CRF neurons under conditions of morphine dependence, male rats received microinjections of fluorescent latex microspheres (Retrobeads) into the LC. Five days later, rats were implanted with morphine or placebo pellets. Seven days post-implantation, a subset of rats was exposed to a single 15-minute swim. Cell counts revealed that c-Fos expression in the CeA was significantly increased in the swim stress groups irrespective of morphine exposure ( $P < 0.05$ ). Morphine treatment further increased c-Fos expression ( $P = 0.05$ ) when compared to placebo irrespective of stress exposure, and compared to morphine alone. Interestingly, more than half of the c-Fos labeled neurons in the morphine dependent stress-exposed group were observed in CRF amygdalar neurons that projected to the LC. These results indicate that morphine exposure increases CRF amygdalar responses to stress and that this increased activation impacts the LC. Such cellular adaptations have important

consequences for increasing brain noradrenergic tone and may predispose chronic opiate users to hyper-arousal, symptom observed in many stress-related psychiatric disorders.

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**319.18/L17. *Role of projections from ventral subiculum to nucleus accumbens shell and ventral medial prefrontal cortex in context-induced reinstatement of heroin seeking***

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Background: In humans, exposure to contexts previously associated with heroin use can provoke relapse. In rats, exposure to heroin-paired contexts after extinction of drug-reinforced responding in a different context reinstates heroin seeking. We previously demonstrated a causal role for projections from ventral medial prefrontal cortex (mPFC) to accumbens shell in context-induced reinstatement of heroin seeking. Because ventral subiculum sends glutamate projections to both accumbens shell and ventral mPFC, we sought to determine whether these projections also contribute to this reinstatement. Methods: We trained rats to self-administer heroin in distinct context and then extinguished lever pressing in a non-drug-associated context; infusions or lever presses were paired with a discrete cue. We then tested the rats in the heroin- and/or extinction-associated contexts under extinction conditions. We first used the retrograde tracer Fluoro-Gold in combination with Fos to assess whether the ventral subiculum→accumbens shell or ventral subiculum→ventral mPFC pathway is activated during context-induced reinstatement of heroin seeking. We then employed an anatomical disconnection procedure to determine whether these projections are functionally involved in this reinstatement. Results: Exposure to the heroin context, but not the extinction context, reinstated lever pressing. Context-induced reinstatement was associated with increased Fos expression in ventral subiculum neurons, including those that project to accumbens shell or ventral mPFC. Contralateral and ipsilateral disconnection of ventral subiculum from accumbens shell decreased context-induced reinstatement. We are currently examining whether disconnection of ventral subiculum from ventral mPFC inhibits this reinstatement. Conclusions: Results suggest that glutamatergic projections from ventral subiculum to accumbens shell play a critical role in context-induced relapse to heroin seeking.

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**319.19/L18. *Machine learning identifies distinct behavioral markers for opiate and stimulant addiction***

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The development of objective markers for addiction can innovate its prevention and treatment. Abundant evidence indicates that drug addiction is characterized by personality, psychiatric, and

neurocognitive manifestations of impulsivity, one of the most viable endophenotypic markers for addiction. However, it is unclear which of its various dimensions would have the highest predictive utility for addiction and whether addiction to different classes of drugs would be characterized by different impulsivity profiles. To address these gaps, we used a machine learning approach, which holds promise for discovering predictive markers of disease. We conducted two machine-learning studies, using different indices of impulsivity as predictors of stimulant and opiate dependence. In Study 1, we recruited current stimulant (cocaine) users and healthy controls (HCs) in USA who completed self-reports of trait impulsivity and neurocognitive tasks of impulsive choice and impulsive action. A machine learning model was fitted on the training set using 5-fold cross validation and we tested its out-of-sample classification accuracy in the test set. The area under the curve (AUC) of the ROC curve was 0.90 in the test set. In Study 2, we tested HCs and individuals with past mono-dependence on heroin or amphetamine, currently in protracted abstinence. The study was done in Bulgaria where poly-substance dependence is still uncommon. Machine learning analyses revealed that the AUC of the ROC curve in the test set was 0.85 and 0.75 for the classification of past heroin and amphetamine dependence, respectively. Heroin and amphetamine dependence were predicted by distinct multivariate personality and neurocognitive impulsivity profiles. Delay discounting predicted stimulant dependence in both studies. Our results demonstrate the promise of machine-learning approaches to extract features predictive of group membership with high degree of accuracy and drug-class specificity and highlight how decision science and advanced statistical methods can inform clinical science. The results suggest that behavioral measures of impulsivity and decision-making can be objective markers of drug addiction. Results suggest that delay discounting may be a viable endophenotypic marker for stimulant (but not opiate) addiction. Our findings suggest that different mechanisms may underlie stimulant and opiate addiction, challenging the unitary account of drug addiction. This line of work may shed light on the development of affordable and easy-to-administer standardized tests that can be used to assess individuals' risk to addiction to different classes of drugs in clinical settings.

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320.01/L19. ***Examining protein synthesis in the nucleus accumbens after withdrawal from extended-access cocaine self-administration***

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During withdrawal from extended-access cocaine self-administration, there is progressive intensification (incubation) of cue-induced cocaine craving that is associated with numerous synaptic adaptations in the nucleus accumbens (NAc). Recent work from our lab suggests these adaptations are maintained by dysregulated local protein translation. Aberrant translation has a profound impact on cellular function and is a key feature in Fragile X syndrome and some other disorders of the nervous system. Treatments to normalize protein synthesis have proven successful in reversing some behavioral and cellular abnormalities in mouse model of Fragile X. Currently, little is known about mechanisms regulating translation in the NAc. Furthermore, the possibility of long-term alterations in translation

following cocaine exposure has been largely uninvestigated and provides an intriguing novel target for therapeutic intervention. We examined the hypothesis that incubation of cocaine craving is associated with dysregulation of protein translation in the NAc. Male Sprague Dawley rats underwent extended-access cocaine or saline self-administration (6hr/10days, 0.5mg/kg/infusion), followed by >4 days of withdrawal. We used <sup>35</sup>S-Met/Cys incorporation to measure protein translation in NAc tissue. Preliminary data indicate that overall translation is not different between cocaine and saline groups, suggesting that translation of only a small subset of proteins may be differentially regulated. Work is underway to compare patterns of translation using bioorthogonal noncanonical amino acid tagging (BONCAT) of newly synthesized proteins to specifically examine translation rates of key synaptic targets either using immunoprecipitation or through an unbiased mass spectrometry approach. We are also comparing the regulation of translation in cocaine versus saline rats by mGluR and NMDA receptors. These studies are the first to characterize how synaptic transmission regulates protein translation in the NAc under basal conditions and whether drugs of abuse cause persistent alterations in the synthesis of proteins linked to addiction.

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320.02/L20. ***Cocaine self-administration alters calcium signaling mediated by NMDA and AMPA receptors in dendritic spines of rat nucleus accumbens neurons***

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Cue-induced cocaine craving intensifies or incubates during withdrawal from extended-access cocaine self-administration. After prolonged withdrawal, incubated seeking is mediated by GluA2-lacking, Ca<sup>2+</sup>-permeable AMPARs (CP-AMPARs) that accumulate in the nucleus accumbens (NAc). The functional consequences of CP-AMPA accumulation have been characterized using whole-cell patch-clamp recordings, but, to our knowledge, no studies have characterized Ca<sup>2+</sup> entry through CP-AMPARs at the level of NAc dendritic spines following the incubation of cocaine craving. In addition to CP-AMPARs, NMDARs play a major role in Ca<sup>2+</sup> signaling and have been increasingly implicated in cocaine-induced neuroadaptations. Here we measured Ca<sup>2+</sup> entry into MSN dendritic spines in the rat NAc following >40 days of withdrawal from saline or cocaine self-administration. We utilized 2-photon microscopy, whole cell electrophysiology, and photo-uncaging of either MNI-L-Glutamate (in the presence of APV) to activate CP-AMPARs or MNI- NMDA to activate NMDARs. We found that cocaine rats exhibited an increase in the proportion of spines responding to MNI-Glutamate + APV, suggesting that some CP-AMPARs are added to spines that did not previously possess them. In addition, cocaine rats demonstrated a significant reduction in the percentage of spines responding to MNI-NMDA photolysis compared to saline control rats. We are evaluating potential explanations for this effect. Support: DA034943 (G.E.S), DA015835, DA009621, DA029099 (M.E.W) and postdoctoral NRSA DA36963 (D.T.C.).

**320.03/L21. Comparison of trafficking mechanisms of calcium-impermeable and calcium-permeable AMPA receptors in rat nucleus accumbens neurons co-cultured with prefrontal cortex neurons**

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Glutamatergic transmission in the nucleus accumbens (NAc) is critical for motivated behaviors, including drug addiction. In an animal model of cocaine addiction called the “incubation” model, rats exhibit progressive intensification or “incubation” of cue-induced cocaine craving during withdrawal from extended access cocaine self-administration. We have shown that this cocaine regimen leads to the accumulation of GluA2-lacking, Ca<sup>2+</sup>-permeable AMPA receptors (CP-AMPA) in the NAc and that these high conductance AMPARs mediate the expression of incubated cue-induced cocaine craving after prolonged withdrawal (Conrad et al., 2008). To understand how this occurs, it would be helpful to know whether different mechanisms regulate the trafficking of CP-AMPA versus the GluA2-containing, Ca<sup>2+</sup>-impermeable AMPA receptors (CI-AMPA) that normally dominate synaptic transmission in the NAc. Because receptor trafficking is difficult to study in vivo, we are using a model system consisting of rat NAc neurons co-cultured with prefrontal cortex (PFC) neurons from enhanced cyan fluorescent protein (ECFP)-expressing mice. The cortical neurons restore excitatory input onto the NAc neurons but can be distinguished based on their fluorescence. NAc medium spiny neurons (MSN) in this co-culture system express high levels of CP-AMPA, recapitulating the state of the NAc after incubation of cocaine craving (Sun et al., 2008). The goal of this study is to compare the regulation of CP-AMPA (homomeric GluA1) and CI-AMPA (GluA1A2) with respect to: 1) rate of receptor internalization and receptor cycling (rate at which tagged surface receptors are replaced by untagged receptors), 2) effect of protein synthesis inhibition on surface receptor levels and cycling, and 3) effect of increasing or decreasing synaptic activity on surface receptor levels and their cycling. So far, our results indicate that: 1) GluA1 and GluA2 exhibit similar rates of constitutive internalization and cycling. 2) Brief protein synthesis inhibition reduces surface levels of both CI-AMPA and CP-AMPA. 3) Following activity blockade (CNQX, 24 h), CI-AMPA, but not CP-AMPA, show increased surface expression and cycling. In contrast, increased activity (bicuculline, 24 h) decreases both CI-AMPA and CP-AMPA surface expression. These studies will better our understanding of mechanisms that shape excitatory transmission in the NAc, which has translational implications related to drug addiction and relapse.

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**320.04/L22. Regulation of protein translation following prolonged withdrawal from extended-access cocaine self-administration with or without cocaine memory retrieval**

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Vulnerability to relapse in cocaine addicts is often attributed to craving evoked by exposure to cues previously paired with the drug. Studies in animal models of addiction have shown that drug memories, upon retrieval, become labile and must undergo reconsolidation to be returned to long-term storage, a process that represents a potential therapeutic target for treating addiction. It is therefore important to understand mechanisms mediating memory retrieval and reconsolidation. It has been shown that de novo protein synthesis is necessary for retrieval and/or reconsolidation of cocaine memories. In addition, numerous studies have implicated mTOR-related signaling, a pathway that regulates protein translation, in cocaine-dependent plasticity. Lastly, previous work from our lab has shown that ongoing protein translation is required to maintain cocaine-dependent synaptic adaptations in the nucleus accumbens (NAc) during prolonged withdrawal. The goal of this study was to determine if the regulation of protein translation, and particular the mTOR pathway, is altered following an extended-access cocaine self-administration regimen that produces progressive intensification, or “incubation”, of cue-induced craving. To study this, we collected NAc tissue from rats that self-administered saline or cocaine and underwent ~5 days of withdrawal. Half the rats in each group experienced a cue-induced seeking test. In our first experiment, we prepared a P2 fraction and evaluated a number of proteins involved in regulation of translation, including two factors involved in translation initiation and elongation, eIF2 and eEF2, respectively. We found retrieval-dependent but treatment-independent increases in phosphorylated and total eIF2 and phosphorylated but not total eEF2. In our second experiment, we prepared NAc synaptoneurosomes to better isolate postsynaptic processes. Analysis of eIF2, eEF2 and other translational regulators is underway.

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320.05/L23 ***Reinstatement to drug-seeking behavior following cocaine, cues, and stress results in synaptic depotentiation of glutamatergic synapses in the nucleus accumbens***

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Relapse to drug-seeking behavior during abstinence, often precipitated by drug re-exposure, cues, or stress, is a major obstacle to long-lasting addiction recovery. Understanding the neurobiology of drug craving and relapse is likely to provide new means for addiction treatment. The nucleus accumbens (NAc), a key target for addictive drugs, is a critical interface of mesolimbic dopamine circuitry with excitatory cortical afferents in regulating motivation and drug seeking. Plasticity of glutamatergic synapses in the NAc is induced by in vivo cocaine and appears to contribute to increased drug-seeking behavior. Repeated experimenter-administered cocaine potentiates synaptic strength in NAc medium spiny neurons (MSNs) following abstinence, while subsequent re-exposure to cocaine (temporarily) reverses this effect. The extent to which this pattern of plasticity occurs following self-administration of cocaine, and whether cue-, or stress-primed reinstatement elicits depotentiation is unknown. Using a combination of cocaine self-administration with NAc whole-cell recordings in an ex vivo brain slice preparation, we examined plasticity at glutamatergic synapses in NAc shell MSNs following reinstatement of drug-seeking behavior precipitated by saline, cocaine, stress, and drug-associated cues.

Mice self-administered 0.5mg/kg/infusion cocaine for 10 days before 8-12 days of extinction training. To reinstate drug seeking, mice were exposed to a priming injection of saline, cocaine (10mg/kg), or yohimbine (2.5mg/kg) prior to, or drug-associated cues during reinstatement testing. As predicted, cocaine-, yohimbine-, and cue-primed animals exhibited greater reinstatement behavior than saline injected animals (228.2±55.9%, 268.0±42.8%, 169.0±21.0%, 95.6±10.2% of extinction responding respectively). In concordance with behavioral data, mice challenged with saline following cocaine self-administration and extinction exhibited elevated AMPA:NMDA ratios (1.53±0.11) in comparison to control animals (0.98±0.08). However, mice that reinstated drug-seeking behavior following a cocaine (1.00±0.04), stress (0.96±0.08), or cue prime (0.92±0.09) displayed de-potentiated AMPA:NMDA ratios down to control levels. Interestingly, AMPA:NMDA ratios were still potentiated in mice that failed to reinstate drug-seeking behavior (1.41±0.08). Collectively, these data indicate a common neurobiological response - AMPAR depotentiation in the NAc shell - to different stimuli that provoke a reinstatement of drug seeking. Future studies will determine the extent to which this response may modulate or mediate drug seeking.

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320.06/L24. ***Optogenetic self-stimulation of the infralimbic-accumbens pathway: Opposing effects of abstinence from repeated cocaine and cocaine re-exposure***

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Mice will actively self-administer optogenetic stimulation of glutamatergic inputs to the nucleus accumbens shell (Stuber et al. 2012; Britt et al. 2012). In drug-naïve animals, optogenetic self-stimulation is largely input-pathway independent, suggesting that glutamate non-discriminately supports behavioral reinforcement (Britt et al. 2012). However, it is unknown how cocaine experience may alter the ability of glutamatergic inputs to the nucleus accumbens shell to promote behavioral reinforcement. We have previously shown that abstinence from repeated cocaine and cocaine re-exposure produce bidirectional synaptic plasticity in the nucleus accumbens shell (Kourrich et al. 2007). Furthermore, while input-specific changes in glutamatergic synaptic function are apparent during cocaine abstinence (Pascoli et al. 2012), the effects of cocaine re-exposure in abstinence on input-specific plasticity is unknown. Therefore, to better understand how input-specific plasticity induced by cocaine experience may alter the ability of excitatory transmission in the NAcSh to reinforce behavior, we compared input-specific optogenetic self-stimulation behavior in animals with different histories of cocaine experience. C57BL/6J mice were infected with channelrhodopsin in either the infralimbic cortex (IL), ventral hippocampus (vHipp), or basolateral amygdala (BLA), and optical fibers were implanted over the NAcSh to allow for selective stimulation of these inputs. Mice were next treated with saline or a cocaine sensitization regimen (15 mg/kg i.p, 5 once daily injections) followed by 10-14 days of abstinence. A spatial optical self-stimulation task was used to assess behavioral reinforcement after cocaine abstinence, or after being re-exposed to cocaine in abstinence. We found that IL-NAcSh self-stimulation was more pronounced in cocaine-abstinent mice compared to drug-naïve controls (10 Hz, 5

ms, 5 s max pulse per entry into the “active” zone). Interestingly, cocaine re-exposure dampened IL-NAcSh self-stimulation behavior to levels of control mice. In contrast, self-stimulation of vHipp-NAcSh inputs was enhanced by cocaine abstinence, and further augmented by cocaine re-exposure. Importantly, these different behavioral effects were paralleled by input-specific changes in synaptic plasticity as measured by whole cell patch clamp recordings in medium spiny neurons. Together, our findings suggest that input-specific changes in plasticity with cocaine abstinence and re-exposure directly modify the reinforcing effects of glutamate in the NAcSh.

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320.07/L25. ***Synaptic depotentiation via mGluR5 activation and AMPAR internalization in the nucleus accumbens shell drives cocaine-primed reinstatement***

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Relapse after periods of drug abstinence is a major obstacle to long-lasting recovery for many addicts. Understanding the neurobiological processes that incite drug craving and drive relapse has the potential to help target our efforts to treat addiction. The nucleus accumbens (NAc) serves as a critical interface of mesolimbic dopamine circuitry with excitatory cortical afferents in the regulation of motivation and drug seeking. Repeated cocaine exposure potentiates synaptic strength in the NAc medium spiny neurons, which is thought to promote addiction-related behavior. However, the present studies tested the hypothesis that reversal of that augmented synaptic strength, or depotentiation, in the NAc shell is the critical factor in relapse. In support of this hypothesis, we used cocaine conditioned place preference (CPP) behavior and ex vivo whole-cell electrophysiology to show that cocaine-primed reinstatement and synaptic depotentiation was disrupted by intra-NAc shell infusion of the tat-GluA23Y “interference” peptide (inhibitor of activity-dependent AMPAR internalization; Ahmadian et al., 2004). Metabotropic glutamate receptor subtype 5 (mGluR5) activation is one mechanism known to promote synaptic depression. Therefore, we investigated whether cocaine-primed reinstatement is driven by an mGluR5-dependent reduction in AMPA-type glutamate receptor signaling. Intra-NAc shell infusion of the mGluR5 antagonist MTEP blocked cocaine-primed reinstatement and corresponding depotentiation, while infusion of the mGluR5 agonist CHPG produced a dose-dependent reinstatement of CPP and depotentiated synaptic strength in the NAc shell. Using an optogenetic approach to look at the role of glutamatergic afferent signaling in the NAc shell, we observed that low frequency blue-light stimulation of the NAc (10 Hz, 5 min, 5ms pulse width) produces reinstatement of CPP in mice infected with AAV-ChR2. Interestingly, this effect was observed when ChR2 was expressed in either the infralimbic cortical or ventral hippocampal projections. This optical stimulation is modeled after electrical stimulation protocols that produce mGluR1/5-dependent LTD (Grueter et al 2010). These findings support a model in which mGluR5-mediated reduction in synaptic GluA2-containing AMPAR function in NAc shell medium spiny neurons can mediate the reinstatement of cocaine-primed behavior.

320.08/L26. ***Bidirectional ethanol-induced synaptic plasticity and reinstatement of place preference following a history of combined ethanol and cocaine exposure***

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Repeated exposure to drugs of abuse induces plasticity at excitatory synapses in the nucleus accumbens (NAc) that are central to the development and persistence of addiction-related behavior. An abundance of data indicates that experience-dependent plasticity in NAc glutamatergic synaptic transmission is primarily expressed via dynamic changes in AMPA-type glutamate receptors (AMPA-Rs), making these receptors a key target for studying how drug experiences modify behavior in models of addiction. In humans, coabuse of drugs is pervasive, with concurrent cocaine and alcohol use being a frequently abused combination; however, to date, knowledge of drug-induced synaptic plasticity is often limited to single-drug regimens. Using a model of conditioned-place preference (CPP), our studies test the hypothesis that co-administration of cocaine and ethanol modulates NAc synaptic plasticity at glutamatergic afferents to MSNs in a way that may explain the potent effects of this drug combination on behavior. In wild-type mice, co-administration of cocaine (7.5 mg/kg ip) and ethanol (2 g/kg ip) as the conditioned stimulus reliably produced a modest conditioned place preference which extinguished rapidly. On re-exposure to ethanol alone (2 g/kg) after extinction, animals showed divergent behavioral responses, with one subpopulation displaying robust reinstatement of preference for the conditioned stimulus ( $580.40 \pm 66.8$  sec CS bias,  $n=16$ ), while another showed dramatic aversion to the conditioned stimulus ( $390.97 \pm 90.4$  sec anti-CS bias,  $n=10$ ) after ethanol primed reinstatement. A subset of mice was sacrificed immediately after the ethanol reinstatement test to examine alterations in synaptic AMPA receptor-mediated transmission in the NAc using whole-cell recordings from MSNs in acute slices. In the NAc shell in animals with a strong preference for the conditioned stimulus, the amplitudes of miniature excitatory postsynaptic currents (mEPSCs) were similar to saline controls. However in animals with inverted preference, mEPSC amplitudes were increased by >16%, suggesting that synaptic plasticity in NAc may underlie aversion to the CS in this population. Our results suggest that re-exposure to ethanol induces bidirectional synaptic plasticity in nucleus accumbens, in parallel with divergent behavioral responses. Ongoing experiments will examine how cocaine modulates this divergence, and whether motor and sensory impairment contribute to bimodal reinstatement after ethanol priming.

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320.09/L27. ***Cellular mechanisms and timing of cocaine-induced synaptic depotentiation in the nucleus accumbens***

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Repeated exposure to cocaine can promote drug-seeking and -taking behavior. In rodent addiction models, enduring changes in glutamatergic synaptic transmission in the nucleus accumbens (NAc) appear to be important in driving this behavior. Repeated exposure to cocaine is known to potentiate AMPAR-mediated signaling in medium-spiny neurons (MSNs) in the NAc shell 10-14 days following the last drug exposure. This increase in synaptic function is reversed (or, “depotentiated”) by a single re-exposure to cocaine. However, the cellular mechanisms and the detailed timing of this drug-evoked plasticity are not well characterized. We investigated the onset and duration of NAc synaptic depotentiation as well as potential underlying mechanisms driving this plasticity, focusing on region-specific changes (shell versus core). Extending previous findings (which focused on NAc shell), we observed that in the NAc core, AMPAR-mediated transmission was augmented following 10-14 d withdrawal, and reversed 24 h following drug re-exposure. In both NAc shell and core MSNs, challenge-induced depotentiation of AMPAR-transmission returned to control levels 5 d following drug re-exposure, indicating that this plasticity is transient. To determine the underlying mechanisms of the drug-induced depotentiation, we employed a novel ex vivo “bath challenge” model of drug re-exposure. Ex vivo re-exposure to cocaine induced depotentiation of AMPAR-mediated synaptic transmission in NAc shell and core MSNs within 30 min of drug exposure. In the NAc, activation of postsynaptic group I metabotropic glutamate receptors (mGluRs) has been shown to promote reduced presynaptic glutamate release probability and increased trafficking of AMPA-type glutamate receptors (McCutcheon et al., 2011; Robbe et al., 2002). Consistent with this, in the NAc shell, prior exposure to the mGluR5 antagonist MTEP blocked the ex vivo cocaine-induced reductions in mEPSC amplitude but not frequency, presumed measures of postsynaptic and presynaptic function respectively. In contrast, in the NAc core, prior exposure to MTEP did not influence the ex vivo cocaine-induced reductions on mEPSC amplitude or frequency. Interestingly, blockade of endocannabinoid signaling using the eCB1 receptor antagonist (SR-141716A), blocked cocaine-induced reductions in mEPSC frequency but not amplitude, suggesting the existence of multiple, independent forms of synaptic plasticity in response to drug re-exposure during abstinence. Using this approach to further delineate mechanisms and timing of drug-induced plasticity will help elucidate the neurobiology of susceptibility to addiction relapse.

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320.10/L28. ***NMDA receptor subtypes control maturation of cocaine-generated silent synapse in nucleus accumbens***

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Exposure to cocaine induces adaptive molecular and cellular changes in the forebrain nucleus accumbens (NAc), leading to subsequent drug craving, drug seeking and drug taking behaviors. Using repeated i.p. injection procedure, we previously demonstrate in the NAc that exposure to cocaine generates AMPA receptor-silent excitatory synapses, which are enriched in GluN2B-containing NMDA

receptors. These cocaine-generated silent synapses are likely immature synaptic contacts that evolve into fully functional synapses after cocaine withdrawal. Similar to the non-contingent procedure, after cocaine self-administration (2 h/session/day x d) we also observed substantial increase in the level of silent synapses in the NAc, enriched in GluN2B-containing NMDARs. Furthermore, the increased level of GluN2B-containing NMDARs returned to the basal levels after 7-day withdrawal, accompanied by the maturation of cocaine-generated silent synapses. In a mouse line in which the CaMKII-binding to GluN2B was compromised, exposure to cocaine generated silent synapse in the NAc, but these silent synapses remain silent after cocaine withdrawal without undergoing the maturation process. These results lead to our hypothesis that GluN2B-containing NMDARs control the maturation of these cocaine-generated silent synapses. Our future experiments will test this hypothesis by determining how GluN2B and its coupled signaling regulate maturation of cocaine-generated silent synapses and whether targeting NAc GluN2B is a strategy to reverse cocaine-induced circuitry remodeling.

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320.11/L29. ***Cocaine self-administration generates silent synapses in thalamus to nucleus accumbens projection***

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Drug Addiction is characterized by maladaptive changes in signaling between brain regions which regulate rewards and motivated behaviors. The paraventricular thalamic nucleus (PVT) is a brain region which sends direct projections to the nucleus accumbens (NAc) and is increasingly being linked to addiction-related behaviors. We sought to characterize the molecular and cellular changes within the PVT-to-NAc pathway in response to cocaine self-administration. We used virally-mediated channelrhodopsin expression in the PVT of rats to isolate fibers from the PVT and recorded from neurons in the NAc shell. We found that cocaine self-administration increases silent synapses within the PVT-to-NAc pathway. Additionally, calcium-permeable AMPARs are present at PVT-to-NAc synapses under basal conditions, but are not additionally recruited to maturing silent synapses. Cocaine self-administration also leads to greater probability of presynaptic vesicle release, which persists through long-term withdrawal. Disrupting PVT neurons that project to the NAc works to inhibit the acquisition of cocaine self-administration. These results characterize an array of cellular and molecular signaling changes along the PVT-to-NAc pathway in the context of cocaine exposure and demonstrate that this pathway plays a significant role in the acquisition of cocaine self-administration.

320.12/L30. ***Cocaine exposure alters thalamo-accumbens synapses***

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Dysregulation of the mesolimbic system is a hallmark of the pathophysiology of drug addiction and other reward-related psychiatric diseases. The nucleus accumbens (NAc) is well known as a key biological substrate that modulates the incentive and hedonic properties of drugs of abuse. At least 90% of the neurons in the NAc are GABAergic medium spiny neurons (MSNs). The MSNs, which provide the output of the NAc, can be divided into two classes anatomically and biochemically: one class of MSNs project primarily to the midbrain DA areas and express D1 DA receptors, while the other MSNs project to the ventral pallidum and express D2 DA and A2A adenosine receptors. MSNs rest at relatively hyperpolarized membrane potentials; therefore excitatory drive is essential to governing the output of the NAc and subsequent complex behavioral outcomes. The prefrontal cortex, ventral hippocampus, and basolateral amygdala, provide major excitatory inputs to the NAc and each have demonstrated relevance to drug exposure and subsequent behavioral abnormalities. However, the midline thalamic nuclei (thal) also send dense glutamatergic projections to the NAc core, but little is known about how these synapses may subserve conditioned reward-related behaviors. To gain a better understanding of the physiology and plasticity at thal-NAc synapses we virally expressed channel rhodopsin in the midline thalamic nuclei of D1-tdtomato BA transgenic mice and performed targeted whole-cell patch-clamp electrophysiology in the NAc core. These experiments revealed that under basal conditions, thal-D1(+) and thal-D1(-) exhibit differential synaptic properties as assessed by ratiometric measures of ionotropic glutamate receptor (NMDAR and AMPAR) function. We then proceeded to assess the consequences of in vivo cocaine exposure on the thal-NAc circuit, following 2 weeks of abstinence from cocaine exposure. These experiments uncovered adaptations at both thal-D1(+) and thal-D1(-) synapses that are likely important for the behavioral changes observed at this stage of cocaine exposure. These novel findings point towards new potential avenues for DBS- or pharmacotherapy-assisted drug addiction treatments.

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320.13/L31. ***Corticostriatal LTP is modulated by direct pathway co-release of dynorphin***

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Synaptic plasticity adjusts behavior adaptively in the case of skill learning, or maladaptively in the case of addiction. Just as dopamine plays a critical role in synaptic plasticity underlying normal skill learning and addiction, endogenous and exogenous opiates modulate learning and addiction related striatal plasticity. While the effect of opioids on LTD has been characterized, their effect on LTP remains unknown. This study investigates the effect of opioid neuropeptides co-released by direct pathway (D1) MSNs on corticostriatal LTP. We cross Ai32 female mice, which contain genes for channel rhodopsin 2

and EYF downstream of loxP-flanked STOP cassette, to Tg(Drd1a-cre) EY217 male mice. This generates Cre-positive offspring with optically-excitabile D1-MSNs. In striatal brain slice from these offspring, we supply blue light through a 40x submersion objective to drive endogenous co-release from the D1-MSN population coincident with theta-burst stimulation of cortical afferents. First, we demonstrate that this afferent theta-burst stimulation (10.5Hz with 50Hz intra-burst) evokes corticostriatal LTP using the whole cell patch recording technique. Subsequently we show that optical activation of D1-MSNs during induction impairs LTP. We hypothesize that the opioid neuropeptide dynorphin, co-released exclusively by D1-MSNs, is responsible for this reduced TBS LTP. Dynorphin-activated kappa opioid receptors reside presynaptically on dopaminergic afferents and are capable of negatively regulating dopamine release, previously demonstrated to be essential for LTP in dorsomedial striatum. In support of this hypothesis, we demonstrate full rescue of LTP induced with optical activation of D1-MSNs by bath application of the kappa opioid receptor antagonist nor-Binaltorphimine dihydrochloride (1 $\mu$ M). Our findings illustrate a physiological phenomenon whereby heightened D1-MSN activity can regulate corticostriatal plasticity. Ongoing experiments will establish whether this indeed functions through regulation of dopamine availability, and will address whether LTP modulation by D1-MSN co-release differs between direct and indirect pathway MSNs. Our findings have important implications for learning in addictive states marked by elevated direct pathway activation.

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320.14/L32. ***Incubation of methamphetamine craving is associated with selective increases in expression of BDNF and TrkB, glutamate receptors, and epigenetic enzymes in cue-activated Fos-expressing dorsal striatal neurons***

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Cue-induced methamphetamine seeking progressively increases after withdrawal (incubation of methamphetamine craving) but the underlying mechanisms are largely unknown. We determined whether this incubation is associated with alterations in candidate genes in dorsal striatum (DS), brain area implicated in cue- and context-induced drug relapse. We first measured mRNA expression of 24 candidate genes in whole DS extracts after short (2 d) or prolonged (1 month) withdrawal in rats following extended access methamphetamine or saline (control condition) self-administration (9-h/day; 10-days). We found minimal changes. Next, using FACS, we compared gene expression in Fos-positive dorsal striatal neurons, which were activated during ‘incubated’ cue-induced drug-seeking tests after prolonged withdrawal, with non-activated Fos-negative neurons. We found significant increases in mRNA expression of immediate early genes (IEGs: Arc, Egr1), Bdnf and its receptor (Trkb), glutamate receptor subunits (Gria1, Gria3, Grm1), and epigenetic enzymes (Hdac3, Hdac4, Hdac5, GLP, Dnmt3a,

Kdm1a) in the Fos-positive neurons only. Using RNAscope® to determine striatal sub-region and cell-type specificity of the activated neurons, we measured co-labeling of Fos with Drd1 and Drd2 in three DS sub-regions. Fos expression was neither sub-region nor cell-type specific (52.5% and 39.2% of Fos expression co-labeled with Drd1 and Drd2, respectively). Finally, we found that DS injections of SCH23390, D1-family receptor antagonist known to block cue-induced Fos induction, decreased 'incubated' cue-induced methamphetamine seeking after prolonged withdrawal. Results demonstrate a critical role of DS in incubation of methamphetamine craving and that this incubation is associated with selective gene-expression alterations in cue-activated D1- and D2-expressing DS neurons.

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320.17/L35. ***Neuroplasticity associated with individual sensitivity to a natural reward following cocaine exposure***

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Withdrawal from psychostimulants such as cocaine and amphetamine has been associated with reduced motivation for natural rewards in both rats and humans. Rats exhibit individual differences in their consumption of naturally rewarding sucrose. There is evidence to suggest that rats with a high preference for sweet substances differ from rats with a low preference in their response to psychostimulants. However, potential difference between these groups in neuronal plasticity in the NAc in response to cocaine has yet to be fully explored. This study examined the effect of a 'binge' cocaine treatment on the motivation to achieve sucrose reward and associated changes in neuronal excitability in the NAc shell of these animals. Rats were trained to respond for sucrose reward on a progressive ratio (PR) schedule of reinforcement and divided into high and low responders based on their breakpoints. Breakpoints were then measured over 5 days of saline or 'binge' cocaine treatments (3 daily injections at 1 hour intervals, 15mg/kg i.p.) and for 2 days after the termination of drug delivery. Following the final PR session, slices containing NAc were prepared and whole-cell patch clamp recordings were taken in the NAc shell. In cocaine treated rats, breakpoints were reduced during the 5 days of cocaine treatment and up to 2 days following the termination of treatment. No difference in the magnitude of the reduction was seen between the high and low responder groups. In saline treated rats breakpoints remained stable for the duration of the experiment. Whole-cell patch clamp recordings from medium spiny neurons in the NAc shell indicated a pattern of differences between the high and low responder groups on measures of action potential firing and excitatory synaptic strength. These results demonstrate that exposure to the same cocaine regime reveals broad variability in excitability of NAc neurons associated with individual motivation for a natural reward. We are now exploring whether individual sucrose preference in the absence of cocaine similarly co-varies with measures of membrane and synaptic excitability.

**320.18/L36. *Reversal of morphine-induced cell-type specific synaptic plasticity in the nucleus accumbens shell blocks reinstatement***

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Repeated exposure to drugs of abuse such as cocaine, induces plasticity at excitatory synapses in the nucleus accumbens (NAc). However, little is known of opiate-induced synaptic plasticity in NAc and its relevance to drug-seeking. Medium spiny neurons (MSNs), the principal NAc cell-type, typically express either the dopamine receptor 1 (D1-MSNs) or dopamine receptor 2 (D2-MSNs), the presence of which plays a role in determining cell physiology and contribution to drug-related behaviors. To identify morphine-induced plasticity in glutamatergic signaling in the NAc, we used BAC transgenic mice expressing fluorescent proteins under the control of the D1R or D2R promoter, and a model of morphine sensitization to identify adaptations in glutamatergic signaling within the NAc shell (and core) following 10-14 d withdrawal. In the NAc shell, AMPAR/NMDAR (A/N) ratios and the amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs) are increased in D1R- but not D2R-MSNs, indicating synaptic potentiation in the D1R-MSNs. To test for morphine-induced changes in the subunit composition of synaptic AMPARs in D1R-MSNs, we assessed current-voltage relationships of evoked EPSCs and the effect of bath application of 1-naphthylacetylsperimine (Naspm), a selective blocker of GluA2-lacking AMPA receptors. The rectification index increased and Naspm decreased evoked EPSC amplitude in neurons recorded from the morphine-exposed mice, indicators of the presence of GluA2-lacking AMPARs. Given the morphine-induced changes in mEPSC frequency, we used paired-pulse stimulation to test for changes in glutamate release probability, we find significant increases and decreases in release probability within D1R-MSNs and D2-MSNs respectively. We next explored whether reversal of this plasticity is able to prevent drug-associated behavior using a conditioned place preference model. We found that repeated administration of the antibiotic, Ceftriaxone, which up-regulates expression of the glutamate transporter GLT-1, during withdrawal reversed increases in D1-MSNs AMPAR signaling, enhanced signaling in D2-MSNs, and blocked reinstatement of morphine place preference. Furthermore, using an AAV viral vector expressing hM3Dq (Gq) DREADD under control of the glial fibrillary acidic protein (GFAP), we demonstrated that repeated activation of DREADD-mediated signaling in the NAc shell during withdrawal attenuates reinstatement of morphine place preference. These data suggest that cell-type specific adaptations in NAc shell MSN synaptic strength are critical pathophysiological mechanisms underlying morphine-associated behavior.

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**320.19/L37. *Drebrin signaling mediates opiate-induced plasticity in the nucleus accumbens***

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Opiate addiction has dramatically increased, becoming a worldwide epidemic with great societal and financial burdens. Drug addiction, defined as a chronic relapsing disease, involves the 'rewiring' of the brain through long-term changes, such as structural plasticity, in several key regions of the mesolimbic dopaminergic pathway. There is a great deal of evidence demonstrating that chronic psychostimulant exposure increases the number of dendritic spines on medium spiny neurons in the nucleus accumbens (NAc). In contrast, exposure to opiates, such as morphine and heroin which similarly induce behavioral sensitization, leads to a decrease in dendritic spine density. However, there are minimal data regarding the cellular neurobiology that regulates this opiate-induced plasticity. Following both morphine sensitization and heroin self-administration there is decreased expression of the actin binding protein drebrin in the NAc. This decrease in drebrin results from an increase in HDAC2 expression and binding at the promoter of the transcriptional start site, accompanied by a decrease in pan-H3 acetylation. Overexpression of drebrin blunted the development of morphine sensitization (5 mg/kg, i.p.) and attenuated the expression of sensitization following morphine challenge (2.5 mg/kg, i.p.) compared to HSV-GFP controls. In order to determine the role of drebrin in drug relapse, animals were trained to self-administer heroin (0.02 mg/kg/inf). Interestingly, following heroin self-administration, overexpression of drebrin in the NAc significantly decreased responding during heroin-primed reinstatement (0.25 mg/kg, s.c.), but not cue-induced reinstatement. Finally, overexpression of HDAC2 leads to a potentiation of behavioral response to low doses of morphine. Taken together, these data suggest that epigenetic regulation of drebrin is functionally regulated following exposure to opiates and may be a key molecular mechanism underlying opiate-induced behavioral plasticity.

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320.22/L40. ***Dopamine-acetylcholine interplay in the basal ganglia modulates AMPA glutamate receptors and behavior***

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Dopamine (DA) and acetylcholine (ACh) converge onto the protein kinase A (PKA) pathway in medium spiny neurons of the striatum and pyramidal output neurons of the medial prefrontal cortex (mPFC) to control the excitability of these neurons and thus behavior, although underlying molecular mechanisms are less clear. Here we measured phosphorylation of AMPA receptors (AMPA receptors) at a PKA site (S845) as an activation indicator of AMPARs in adult rat brains in vivo to explore how DA and ACh interact to modulate AMPARs and behavior. In a series of pharmacological experiments, we characterized GluA1 S845 responses to DA D1 receptor (D1R), D2 receptor (D2R) or muscarinic M4 receptor (M4R) agents in DA responsive regions (striatum and mPFC). The S845 responses support a local multitransmitter interaction model in which D2Rs inhibited an intrinsic inhibitory element mediated by M4Rs to enhance the D1R efficacy in modulating AMPARs. Consistent with this, selective activation of M4Rs through a positive allosteric modulator (PAM) resumed the cholinergic inhibition of D1Rs. In addition, D1R and D2R coactivation recruited GluA1 and PKA preferentially to extrasynaptic sites. Behaviorally, the M4R

PAM inhibited the motor responsivity to co-injected D1R/D2R agonists. Our in vivo data support the existence of differential DA-ACh balances in the striatum and mPFC which actively modulate GluA1 AMPARs and behavioral sensitivity to changing DA input. central signaling pathway in these balances involves PKA-dependent and S845-regulated trafficking of GluA1.

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**321.06/M4. *Pharmacological inhibition of monoacylglycerol lipase systemically and centrally in the amygdala and visceral insular cortex prevents establishment of naloxone-precipitated morphine induced conditioned place aversion in rats***

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Enhancement of endocannabinoid activity, through pharmacological inhibition of the catabolic enzyme fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (MAGL), has been shown to reduce somatic symptoms of morphine withdrawal in mice (Ramesh et al., 2011; Ramesh et al., 2013). However, the effect of such treatments on the affective properties of morphine withdrawal has not been investigated in rats. The conditioned place aversion paradigm represents an animal model capable of assessing the ability of pharmacological manipulations to alter affective morphine withdrawal. Specifically, morphine induced conditioned place aversion is produced when naloxone is administered 2 hr following single exposure to high dose of morphine (Parker and Joshi, 1998). Our experiments demonstrate that systemic pretreatment with the monoacylglycerol lipase (MAGL) inhibitor, MJN110 (which selectively elevates the endocannabinoid 2-arachidonoylglycerol - 2-AG), but not the fatty acid amide hydrolase (FAAH) inhibitors, URB-597 and PF-3845 (which selectively elevate the endocannabinoid anandamide - AEA), interferes with the establishment of a naloxone-precipitated conditioned place aversion; a model of affective morphine withdrawal. Furthermore, central administration of MJN110 to regions of the amygdala or to the visceral insular cortex (VIC) also prevents the establishment of the place aversion. The effect of MJN110 to interfere with withdrawal was reversed with pretreatment of the CB1 antagonist AM251, and MJN110 administration alone did not possess rewarding or aversive properties in the place conditioning paradigm. Ultimately, these findings suggest pharmacological treatments that elevate 2-AG acting at the CB1 receptor may be useful in reducing the aversive effects of morphine withdrawal.

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**321.08/M6. *Distinct neuronal ensembles in rat infralimbic cortex control food reward memories and extinction memories***

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Animals can be trained to perform an operant response to receive a reward and then to extinguish the learned response when the reward is withheld. Reward memories and extinction memories are thought to be distinct and are likely encoded by different patterns of sparsely distributed neurons called ‘neuronal ensembles’. In a previous study, we found elevated Fos immunoreactivity in the infralimbic cortex in rats exposed to 2 days of extinction training, suggesting that neuronal ensembles within the infralimbic cortex encode extinction memory. To test this hypothesis, we first trained food-restricted transgenic cfos-lacZ rats to lever press for palatable food pellets for 7 days (60 min/day). Two groups of rats were subsequently exposed or not exposed (levers retracted) to 2 days of extinction training (60 min/day). On induction day, one day later, all rats were exposed to a brief induction session (15 min) under extinction conditions to induce Fos. After 75 additional minutes, we selectively inactivated infralimbic neuronal ensembles associated with either the food reward memory (no extinction group) or the extinction memory (extinction group) using the Daun02 inactivation procedure to determine the effects of inactivating ensembles on food seeking. We hypothesized that the ‘food reward ensemble’ is reactivated on induction day when there was no prior extinction training and that the ‘extinction ensemble’ is reactivated after 2 days of prior extinction training. Two days after Daun02 inactivation, rats in the no extinction group had decreased, while rats in the extinction group had increased their lever presses in a brief (15 min) test under extinction conditions. Here, we show that selective inactivation of extinction ensembles impaired extinction recall, while selective inactivation of reward ensembles disrupted reward recall. Results demonstrate that neuronal ensembles encoding reward and extinction memories can intermingle in the same brain area. This research was supported [in part] by the Intramural Research Program of the NIH, NIDA

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321.09/M7. ***Fos-expressing neuronal ensembles in learned behaviors using the Fos-Tet-Cre transgenic rat system***

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Since proposed by Hebb in 1949, evidence supporting the hypothesis that learned associations are encoded in sparsely distributed ‘cell assemblies’ (neuronal ensembles) has primarily been based on correlations between in vivo electrophysiological firing or two-photon calcium-imaging patterns during learning and memory tasks and learning-related post-mortem activity patterns of immediate early genes (IEGs) such as c-fos, arc and zif268. Until recently, the lack of ensemble-targeted approaches has made it difficult to examine whether this sub-population of neurons that is selectively activated during learned behaviors mediates these associations. Current methods target all neurons within a specific region or belonging to a specific cell-type, regardless of whether or not they were selectively activated during learned behaviors. Recently, the Hope lab developed a novel tool—the Fos-Tet-Cre transgenic rat system—that permits recombination of Cre-inducible genes in activated Fos-expressing neuronal ensembles within a 6-h time window following systemic tetracycline injections. The system was

validated using a rat model of context-induced relapse to cocaine seeking using Cre-inducible YFP and Fos-immunoreactivity to assess context-specific reactivation of neuronal ensembles in ventral mPFC. Further, selective inhibition of these context-encoding neuronal ensembles using Cre-inducible halorhodopsin decreased context-induced relapse. We are now pursuing high resolution mapping of neural circuit dynamics in freely behaving Fos-Tet-Cre transgenic rats using a combination of GRIN endoscope-based calcium imaging, two-photon microscopy, and viruses for cell-type and task specific expression of fluorescent reporter proteins.

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321.12/M10. ***The effects of chronic adolescent toluene on behavioral flexibility and intrinsic excitability of mPFC neurons***

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Psychoactive inhalants such as toluene induce changes in neural connectivity, electrophysiology, and behavior that are similar to those produced by classic drugs of abuse. Toluene and other abused inhalants are predominantly used by adolescents, and exposure to these compounds may contribute to behavioral problems associated with drug use such as compulsivity and reduced executive control. While use of volatile solvents is associated with deficits in fronto-cortical dependent behaviors in humans, studies of solvents using well-validated animal models of behavioral flexibility are lacking. To address this issue, we treated male, adolescent Sprague-Dawley rats (P39 at first exposure) to ten minute, twice daily exposures to toluene (~5400PPM) for five consecutive days. After reaching adulthood (P60) rats were trained in operant boxes to lever-press in response to a light cue for 20% sweetened condensed milk. We then tested behavioral flexibility using an attentional set-shifting task followed by a reversal learning task. As predicted, rats that experienced chronic toluene exposure during adolescence took longer to learn the initial reward contingencies during training. Surprisingly however, the toluene exposed group reached criteria in significantly fewer trials compared to air treated controls during the set-shifting task, a result driven by a marked reduction in perseverative errors. There were no significant differences between the two groups during the reversal learning task. Lesion and pharmacological studies have identified two subdivisions of the frontal cortex, the medial prefrontal cortex (mPFC) and orbital frontal cortex (OFC), as regions responsible for attentional set-shifting and reversal learning, respectively. We thus hypothesized that the behavioral alterations observed in toluene treated rats could be due to altered mPFC neuron electrophysiology and dendritic spine morphology. To test this idea, we performed current-clamp electrophysiology and spine imaging on separate cohorts of adult animals treated with toluene vapor during adolescence. To date, results from electrophysiology experiments reveal that chronic adolescent toluene exposure enhances measures of excitability including hyperpolarized action potential threshold and reduced after hyperpolarization potential. These data suggest that toluene selectively alters mPFC-dependent behavior, which could be result of enhanced neuronal excitability in this region. Understanding the effects of chronic adolescent

toluene could provide further insight into the neural basis of both drug addiction and behavioral flexibility Supported by NIDA R01 DA013951.

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322.01/M13. ***Menthol attenuates respiratory irritation responses to cigarette smoke and oral nicotine aversion in C57BL/6 mice: Role of TRPM8***

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Addition of menthol to cigarettes may be associated with increased initiation of smoking, however, the mechanisms underlying this association are not known. Menthol, possibly through its effects on TRPM8 ion channels in cold-sensing peripheral sensory neurons, is known to inhibit the sensation of irritation elicited by respiratory irritants activating TRPA1, TRPV1 and other chemosensory irritant receptors. However, it remains unclear whether menthol modulates cigarette smoke irritancy and nicotine absorption during initial exposures to cigarettes, thereby facilitating smoking initiation. Using plethysmography in a C57Bl/6J mouse model, we examined the effects of L-menthol, the menthol isomer added to cigarettes, on the respiratory sensory irritation response to primary smoke irritants (acrolein and cyclohexanone) and smoke of reference cigarettes. We studied L-menthol's effect on blood levels of the nicotine metabolite, cotinine, immediately after exposure to cigarette smoke. We also examined the effects of menthol on oral nicotine aversion in mice in the two bottle drinking paradigm. L-menthol suppressed the irritation response to acrolein with an apparent IC<sub>50</sub> of 4 ppm. Suppression was observed even at acrolein levels well above those necessary to produce maximal response. Respiratory irritation caused by cigarette smoke was significantly suppressed by L-menthol even at smoke concentrations as high as 30 mg/m<sup>3</sup>. L-menthol's effects were abolished by treatment with a selective inhibitor of TRPM8, the neuronal cold/menthol receptor. Inclusion of menthol in the cigarette smoke resulted in a ~1.5-fold increase in plasma cotinine levels over those observed in mice exposed to smoke without added menthol. In the two bottle drinking paradigm addition of menthol reduced oral aversion to nicotine. This effect was reversed in Trpm8-deficient mice which showed an aversion to mentholated solutions. These findings document that, L-menthol, through TRPM8, is a strong suppressor of respiratory irritation responses, even during highly noxious exposures to cigarette smoke or smoke irritants, and increases blood cotinine. In addition to its effects on respiratory irritant sensing, menthol also suppressed aversion to oral nicotine, known for its irritating effects and bitter taste. The data suggest that L-menthol, as a cigarette additive, may promote smoking initiation and nicotine addiction. These effects may extend to other tobacco products, including electronic cigarettes and smokeless tobacco products.

322.08/M20. *Se differences in morphine tolerance and reward in “humanized” A118G mice*

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The rewarding and analgesic effects of opiates are mediated primarily by the mu-opioid receptor, of which the A118G single-nucleotide polymorphism (SNP) has the greatest link to addiction potential. Clinical and preclinical studies find the G allele associated with increases in heroin reward and self-administration. Clinically, heterozygous individuals expressing one copy of 118G allele report greater pain and reduced responsiveness to opioid drugs after surgery. Taken together, these results suggest that the G allele may confer a genetic vulnerability to opiate addiction and reward. Given the rise in the use of prescription opiates, it is important to understand how the A118G SNP may alter reward, sensitivity, and tolerance to opiates to better treat patients and to also identify those vulnerable to opiate abuse. The purpose of this study was to assess whether the A118G SNP differentially mediates responsiveness to morphine and whether these responses vary as a function of sex. Male and female mice homozygous for the “humanized” 118AA or 118GG alleles were used to test the hypothesis that 118GG mutant mice are less sensitive to the acute and rewarding effects of morphine and developed tolerance to its antinociceptive effects at a faster rate than 118AA wild-type mice. The rewarding effects of morphine were assessed using a conditioned place preference test (CPP). Sensitivity and chronic tolerance to the antinociceptive effects of morphine were examined using the tail-flick, hotplate, and formalin tests. We find that 118AA (but not 118GG) females developed CPP to morphine. While morphine tolerance was not different between 118AA and 118GG mice there was a main effect of sex driven by differences in initial sensitivity to morphine. Interestingly, a challenge dose of morphine two weeks post testing found the effects of tolerance continued to persist in female, but not in male mice. In addition, many of the sex effects observed were driven by differences between the 118AA males and 118GG females, suggesting a genotype interaction related to sex differences. These results provide further evidence of gender-specific differences between males and females in regards to their sensitivity and tolerance responses to morphine with females exhibiting lower sensitivity to the antinociceptive effects of morphine. In particular, the 118GG females seem to show the least responsiveness to both the antinociceptive and rewarding effects of morphine. Taken together, these data suggest that female 118GG mice may need more morphine to achieve the same degree of antinociception, which could lead to increased susceptibility to opiate abuse.

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322.09/M21. *Differential effects of positive nAChR modulators and AChE inhibitors in rhesus monkeys discriminating nicotine*

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Galantamine, an acetylcholinesterase (AChE) inhibitor and positive allosteric modulator of nicotinic acetylcholine receptors (nAChRs) that has been approved for use as a cognitive enhancer in humans, has recently demonstrated potential as a smoking cessation aid in pre-clinical assays. One way galantamine might serve as an effective therapy for smoking cessation would be by producing nicotine-like effects. In the current study, a nonhuman primate model of subjective effects was used to examine the extent to which galantamine shares effects with nicotine; moreover, the relative contribution of AChE inhibition and positive allosteric modulation of nAChR to the effects of galantamine was examined. Galantamine was studied in addition to donepezil, another AChE inhibitor which has been approved for attenuation of the cognitive deficits associated with Alzheimer's disease, and PNU-120596, a positive allosteric modulator of nAChRs that produces cognitive enhancement in monkeys. Rhesus monkeys (n=5) discriminating nicotine (1.78 mg/kg calculated as the base weight) responded under a fixed ratio 5 schedule of stimulus-shock termination. Nicotine, galantamine, and donepezil dose-dependently increased nicotine-lever responding; the percentage of nicotine-lever responses was a mean of 98% at 1.78 mg/kg of nicotine, 98% at 1.78 mg/kg of galantamine, and 89% at 0.56 mg/kg of donepezil. The ED50 values (95% confidence limits) were 0.41 (0.1-1.74) mg/kg for nicotine, 0.77 (0.46-1.28) mg/kg for galantamine, and 0.20 (0.14-0.29) mg/kg for donepezil. PNU-120596, up to a dose of 1 mg/kg, produced a maximum of 1% nicotine-lever responding. Collectively, these results suggest that AChE inhibition and direct nAChR stimulation result in overlapping subjective effects, whereas positive nAChR modulation does not appear to be sufficient to mimic the subjective effects of nicotine. Supported by USPHS Grant DA25267.

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322.14/M26. ***Opposing effects of group I mGluRs on dendritic spine density in the rat nucleus accumbens***

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The group I mGluRs, mGluR1a and mGluR5, are involved in the development and expression of drug addiction. Manipulation of group I mGluR activity can attenuate behavioral sensitization to drugs of abuse, drug seeking behaviors in models of self-administration, and conditioned place preference, suggesting that modulation of these receptors is a possible avenue for therapeutic treatment. Group I mGluR activity affects both neuronal structure and function in the nucleus accumbens (NAc), which likely plays a role in their effect on addictive behavior. In particular, mGluR5 activity has been associated with synapse elimination in the NAc; however, the role of mGluR1a in structural plasticity in this region is less clear. We sought to compare the effects of mGluR1a versus mGluR5 activation on structural plasticity in the NAc. Rats were given a systemic injection of an mGluR5 positive allosteric modulator (PAM), CDPPB, an mGluR1 PAM, SYN119, or vehicle and were then sacrificed 24 hours later. Neurons in the NAc were ballistically labeled with Dil, and spine densities were determined. Our data revealed an interesting bidirectional effect on spine density, with activation of mGluR5 by CDPPB resulting in

decreased dendritic spine density in both the core and shell of the NAc, but mGluR1a activation by SYN119 increasing dendritic spine density in both of these subregions. While changes in spine density were measured 24 hours after drug administration, changes in actin binding proteins and their regulators were observed on a more rapid timescale. These data suggest that positive modulation of different group I mGluRs results in activation of opposing signaling pathways in the NAc that lead to bidirectional effects on cytoskeletal regulation and dendritic spine density.

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**322.15/M27. *Estradiol facilitation of extended access cocaine self administration in female rats requires activation of mGluR5***

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Females exhibit enhanced responsiveness to the addictive properties of cocaine and several other drugs of abuse. The underlying neural mechanisms that produce this heightened response are not well understood. We have recently found that estradiol decreases dendritic spine density in the core of the female nucleus accumbens, with subsequent enhancement of behavioral sensitization to cocaine, through activation of the metabotropic glutamate receptor mGluR5. The present experiment sought to determine whether mGluR5 activation is also required for the facilitative effects of estradiol on cocaine self administration. To test this hypothesis, ovariectomized female rats treated with estradiol on a 2 days on, 2 days off schedule were initially trained to self administer sucrose pellets (FR1 schedule). Females were then removed from estradiol treatment, implanted with IV catheters, and trained to self administer cocaine (1.5 mg/kg/inf; FR1 schedule). At the completion of cocaine training, females were again restarted on the 2 days on, 2 days off schedule with either oil or estradiol, but with the additional injection of either the mGluR5 antagonist MPEP or vehicle 30 minutes prior to hormone injections. Extended access to cocaine self administration was for 14 days (1.5 mg/kg/inf; 6 hrs per day, FR1 schedule). Females treated with estradiol had significantly higher average daily infusions of cocaine across the 14 days of extended access compared to oil-treated females. This effect of estradiol was completely blocked by MPEP treatment. Furthermore, MPEP treatment alone had no effect on self administration. These data indicate that mGluR5 signaling may be a critical mechanism linking estradiol to enhanced addictive responses in females.

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**322.16/M28. *In female rat nucleus accumbens, the endocannabinoid system mediates the effects of estradiol on psychostimulant responses and structural plasticity***

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Estradiol potentiates psychostimulant responses in females, yet the underlying neurobiological mechanisms remain a mystery. Recently our lab has demonstrated that in female rats, the facilitatory effect of estradiol on cocaine-induced locomotor sensitization required activation the metabotropic glutamate receptor mGluR5, and the cannabinoid receptor CB1R. To search for neurobiological underpinnings to these behavioral effects of estradiol, we investigated structural plasticity within the nucleus accumbens (NAc) core. Similar to the observations regarding locomotor sensitization, pretreatment with the mGluR5 antagonist, MPEP (1mg/kg), blocked estradiol-mediated decreases in nucleus accumbens (NAc) core dendritic spine density. In addition, we demonstrate that the estradiol-mediated decrease in dendritic spine density is attenuated by pretreatment with the CB1R inverse agonist, AM251 (1mg/kg). Collectively our data suggest that the effects of estradiol on psychostimulant behavioral responses and NAc core structure require activation of both mGluR5 and CB1R. These data support our model wherein estradiol binds membrane-localized ER $\alpha$  to transactivate mGluR5 in the female rat striatum, leading to CB1R activation. Experiments are currently underway to definitively determine whether estradiol activation of mGluR5 directly mobilizes endogenous cannabinoids (endoCBs) within the NAc.

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411.01/L1. ***Chronic intermittent pattern of alcohol use promotes degradation of HDAC4 and HDAC5 in the rat striatum and enhances compulsive cocaine self-administration***

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Cocaine addiction is invariably preceded by experiences with legal and decriminalized drugs such as alcohol, nicotine, and marijuana. The biological mechanisms by which prior drug experiences contribute to the pathophysiology of cocaine addiction are poorly understood. To determine how long-term alcohol use affects the molecular and behavioral effects of subsequent cocaine use, we have used a rodent sequential drug-administration paradigm that models the stages of drug use seen in humans. We find that animals with a history of alcohol use have enhanced motivation, persistence, and compulsivity for cocaine self-administration. Chronic alcohol use potentiates the effects of cocaine by inhibiting nuclear histone deacetylase (HDAC) activity in the striatum, a brain region critical for addiction-related reward. HDAC inhibition resulted in global acetylation of H3 and H4 lysine residues in the nucleus accumbens, and enhanced cocaine-induced expression of  $\Delta$ FosB, an immediate early gene splice variant found to have a causal role in the transition from recreational to habitual cocaine use. We find that alcohol withdrawal, but not acute alcohol, promotes degradation of the class IIa histone deacetylases HDAC4 and HDAC5, and that this effect is rescued by the selective proteasomal inhibitor lactacystin. Moreover, an intermittent pattern of alcohol use had a more robust effect on nuclear HDAC activity and cocaine-induced compulsivity than did continuous, uninterrupted alcohol use. Together, our findings show that an intermittent pattern of alcohol consumption enhances vulnerability to cocaine addiction by proteasome-mediated inhibition of the HDAC4 and HDAC5. These findings provide new insight into

the mechanisms by which the pattern of alcohol usage increases vulnerability to addiction and suggests new approaches to clinical interventions.

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411.02/L2. ***Effects of suvorexant, a dual orexin receptor antagonist, on motivation for and affective processing of self-administered cocaine in rats***

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Orexins (hypocretins) are neuropeptides produced within lateral hypothalamic regions that project to substrates involved in reward and emotion processing. Orexin peptides bind to and exert downstream effects via two predominantly excitatory G-protein coupled receptor subtypes (Ox1R and Ox2R). Multiple lines of evidence have demonstrated functional roles of orexin signaling in arousal, sleep/wakefulness, and motivated consummatory behavior for natural and drug rewards. Moreover, previous work has shown that selective pharmacological antagonism of Ox1Rs either systemically or directly into the ventral tegmental area disrupts place conditioning for drugs of abuse and reduces effortful behavior associated with cocaine self-administration. Suvorexant, dual orexin receptor antagonist (DORA), recently received FDA approval to treat insomnia. The present study was thus designed to assess potential therapeutic efficacy of suvorexant in an animal model of substance abuse. To accomplish this, adult male Sprague-Dawley rats were trained to self-administer cocaine (~0.355 mg/kg/inf) first under a fixed-ratio 1 schedule of reinforcement for 2 h across 14 daily sessions and then under a progressive-ratio schedule of reinforcement for 4 h across 10 daily sessions. A within-subjects counterbalanced design was used, and animals received pretreatment of suvorexant (3.0, 10.0, or 30.0 mg/kg; 100 µL) or vehicle (100% DMSO; 100 µL) prior to an experimental progressive-ratio scheduled session but following consecutive fixed- and progressive-ratio scheduled sessions. In addition to operant responses, ultrasonic vocalizations (USVs) were recorded to assess changes in affective state during progressive-ratio cocaine self-administration, and *in vivo* fast-scan cyclic voltammetry was used in a cohort of mice to assess cocaine-induced changes in dopaminergic transmission within the ventral striatum. Analyses revealed that suvorexant dose-dependently reduced the number of infusions earned and total correct responses performed relative to respective previous day behavior. Preliminarily, USV data suggest that suvorexant attenuates positive affect associated with cocaine self-administration, and fast-scan cyclic voltammetry data suggest that suvorexant decreases cocaine-induced extracellular dopamine release in the ventral striatum. Taken together, results from the present study suggest that suvorexant, a clinically-used DORA, may contribute in part to a substance abuse therapy by altering reward and affective processing of self-administered cocaine.

**411.06/L6. Preventing cocaine-induced transitions to habit-based behavior through inactivation of the basolateral amygdala**

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core feature of substance use disorders reflects persistent addictive behavior, despite knowledge of the adverse consequences associated with it (e.g., loss of friends and family, ill health). This maladaptive habitual behavior (i.e., continuing to perform an action that leads to a bad outcome) can be modeled in rats using the reinforcer devaluation paradigm. Rats were trained to perform two actions for the receipt of distinct reinforcers (e.g., lever 1→sucrose; lever 2→polyucose). Following training, rats received prefeeding treatment with one of the outcomes (e.g., unlimited sucrose access), which served to devalue it. As a result of this manipulation, drug naïve control rats showed a spontaneous reduction in responding to the lever associated with the devalued outcome (e.g., lever 1). In contrast, rats with a history of chronic cocaine exposure continued to respond for the devalued outcome. Next, we examined whether we could prevent this cocaine-mediated transition to habit-based behavior. To achieve this we inactivated the basolateral amygdala (BLA) (via microinfusions of baclofen / muscimol) during cocaine exposure, as this region is particularly vulnerable to the detrimental effects of cocaine. Notably, inactivation of the BLA subsequently prevented the transition to habitual behavior, as evidenced by the ability of these rats to correctly modify their responding based on reductions in outcome value. Thus, through this inactivation procedure we were able to prevent cocaine-induced habit-based behavior from occurring.

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**411.07/L7. Selectivity of pilocarpine and tacrine effects on cocaine- and food-reinforced responding in rats**

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Drugs that alter activity of the brain's cholinergic system can cause large and persistent reductions in behaviors maintained by drug- and natural- reinforcers. Notable examples include muscarinic agonists such as pilocarpine and cholinesterase inhibitors such as tacrine. The present study compared the effects of pilocarpine and tacrine on cocaine- and food- reinforced behavior, and spontaneous scored behaviors in rats, with the overall goal of understanding the selectivity of actions on drug-reinforced behavior. METHODS: Animals acquired responding for either cocaine or liquid food reinforcement, and were gradually shaped to work under a fixed-ratio-5 (FR-5) schedule, with 2 second time out, during two-hour multiple-component sessions. Pilocarpine or tacrine was injected intraperitoneally at low, intermediate, or high doses (0.66 (pilocarpine only), 1.0, 3.2, or 10 mg/kg). RESULTS: Pilocarpine

attenuated self-administration of low- and intermediate doses of cocaine, but was ineffective at modifying responding for high-dose cocaine (effective-dose-50 [ED50] values of 1.19 and 4.49 mg/kg, respectively). Responding for low amounts of liquid food was also attenuated by pilocarpine (ED50 value of 9.80), with greater liquid food amounts unaffected. These results correspond to 3.77-fold greater selectivity for pilocarpine in attenuating low-dose cocaine, relative to low amounts of liquid food. For low-dose cocaine and small amounts of liquid food, tacrine was also more effective in attenuating drug-reinforced behavior, relative to liquid food (ED50 values of 1.69 and 4.67), corresponding to a 2.76-fold greater selectivity for drug reinforcement. In contrast, tacrine was more effective in attenuating intermediate and high levels of liquid food reinforcement, relative to comparable amounts of cocaine (corresponding to 5.03- and 2.87- fold greater selectivity for liquid food, respectively). The combination of pilocarpine and tacrine attenuated either low-dose cocaine or liquid food reinforced responding with similar potency (ED50 values of 1.52 and 1.56, respectively). CONCLUSION: When compared to a natural, non-drug reinforcer, both pilocarpine and tacrine exhibit good selectivity for attenuating low-dose cocaine. This selectivity is lost for behavior reinforced by a higher, intermediate dose of cocaine.

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411.08/L8. ***Hypocretin 1 receptor blockade preferentially reduces high effort responding for cocaine without promoting sleep***

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Recent evidence suggests that blockade of the hypocretin receptor 1 may act as a useful pharmacotherapy for cocaine abuse. In these studies we investigated the extent to which various doses of a hypocretin receptor 1 antagonist, SB-334867, affects cocaine self-administration across a range of effort requirements, and tested if these doses produce sedative effects. First, we trained animals to self-administer one of three doses of cocaine on a progressive ratio schedule, and then tested the effects of one of three doses of SB-334867. Responding for cocaine was then characterized with a novel two phase analysis that segregates features of relatively high and low effort requirements across the progressive ratio session. In another set of experiments we tested the sleep promoting effects of the same doses of SB-334867. Our data indicate that blockade of hypocretin receptors selectively reduces effortful responding for cocaine at levels that do not promote sedation. These findings lend further support for the use of hypocretin receptor 1 antagonists in the treatment of cocaine addiction.

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411.09/L9. ***Cocaine vs food choice under a dependent schedule***

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growing literature has demonstrated that alternative reinforcers can modulate the reinforcing effects of drugs of abuse. Herein, we assessed the relative value of an alternative reinforcer (sucrose pellet) against drug reward (cocaine), using dependent schedule. Unlike other choice paradigms, a dependent schedule maintains equivalent experience with both reinforcers to help control for any systematic bias that may develop from differential sampling of the alternatives during choice opportunities. Male Sprague Dawley rats were initially magazine shaped and then trained to lever press for sucrose pellets on a FR1 to FR5 schedule. Following FR5 lever training, rats were placed on a response chain where the onset of the houselight signaled a contingent head entry (orienting) response into the magazine, which turned off the houselight and extended the response levers. After head entry and lever training, rats were catheterized. Rats were then trained on an FR1 to FR5 schedule for 1.0 mg/kg/infusion of cocaine. Next, rats were allowed to earn five sucrose pellet reinforcers and five cocaine reinforcers (1.0 mg/kg/infusion) on a FR5, where presentation of the associated lever (cocaine or food) was randomly presented alone. Finally, rats were placed on a dependent schedule, where they were allowed to earn three sucrose pellet reinforcers and three cocaine reinforcers per block; setup of each reinforcer (sucrose or cocaine) was randomized across trials within each block. The dose of cocaine increased across blocks (0, 0.03, 0.1, 0.3, and 1.0 mg/kg/infusion), and each block was accompanied by a distinct tone that served as a discriminative stimulus. While there were individual differences in the degree of relative preference for cocaine, the results demonstrate that, in all animals, cocaine preference was dose-dependent, increasing with increases in dose. Overall, the results indicate that sucrose and cocaine preference is relative. Furthermore, utilizing dependent schedule allows for the systematic study of the relative reinforcing effects of natural and drug reinforcers without confounding differential experience with either reinforcer during choice. Further study of choice between drugs of abuse versus natural reinforcers using dependent schedule will allow for better understanding of the neurobehavioral mechanisms that drive substance abuse.

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411.11/L11. ***Short and extended cocaine abstinence in behaviorally sensitized animals elicits differential firing patterns in medial prefrontal cortex and nucleus accumbens***

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Behavioral sensitization refers to the progressive augmentation of behavioral responses to psychomotor stimulants (Stewart and Badiani, 1993 Behav Pharmacol 4:289-312) and is thought to be key process underlying drug addiction (Robinson and Berridge, 1993 Brain Res. Rev. 18 247-291). Behavior is thought to be controlled by cortical structures that regulate the function of subcortical motor structures. In this study, chronically implanted microelectrode bundles were used to record medial prefrontal cortex and nucleus accumbens activity in behaving rats in response to the first and last day (challenge) injection of cocaine. We assessed electrophysiological changes in two key projections: the prelimbic (PrL)- nucleus accumbens core (NAc) projection and the infralimbic (IfL)-nucleus accumbens shell (NAs) projection. Rats were tested in a 10- or 32-day sensitization paradigm in which they received an i.p. injection of

cocaine (15 mg/kg on recording days 1, 7, and the challenge day; 3 mg/kg on days 2-6) for 7 consecutive days and then on the challenge day after a 2- or 25-day withdrawal period. The activity of individually isolated single neurons was recorded simultaneously with local field potentials (LFPs) in a sound-attenuated, open-field chamber while the animals moved freely. Consistent with the sensitization paradigm, locomotor and focused stereotyped behavior increased with longer abstinence periods. Cocaine-induced changes in neuronal firing patterns (e.g., spike bursting) and LFPs (e.g., behavioral modulation of oscillatory activity) were analyzed within and between recording days. We also assessed region-specific correlated firing and LFP coherence between regions. Preliminary assessment of electrophysiological data indicates differential patterns of neuronal signaling between PrL-NAc and lI-NAc projections related to short and extended abstinence periods. Identifying the mechanisms underlying behavioral sensitization to cocaine and other psychomotor stimulants can aid in learning how to regain forebrain control of behavior lost to abuse of these substances.

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411.12/L12. ***Optogenetic stimulation of red nucleus glutamate neurons inhibits cocaine self-administration in mice***

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The red nucleus (RN) is a nucleus adjacent to the ventral tegmental area (VTA) in the midbrain and considered as a part of the motor control system. RN gives rise to the rubrospinal tract that sends fibers to the contralateral side at the VTA level, forming the ventral tegmental decussation, and projects to the cervical spinal cord. Therefore, it has been long considered that the nerve fibers traveling across to the contralateral VTA are merely descending fibers of passage of the RN. Little is known as to whether these decussation fibers synapse on VTA neurons directly or indirectly via collateral branches. In the present study, we used PHA-L anterograde tracing and FluoroGold retrograde tracing methods, and found that there are direct neural projections from the RN to the contralateral VTA, suggesting possible interactions between RN and VTA neurons. RN neurons are predominantly glutamatergic (GLUergic), with a small population of GABAergic neurons. GLUergic neurons in other brain regions such as prefrontal cortex, amygdala or ventral hippocampus (vHipp) play a role in the regulation of drug-seeking and drug-taking behaviors modulated by the VTA and its projections. The question thus arises- do RN GLUergic neurons similarly regulate drug-seeking and drug-taking behaviors? Optogenetic technology was used to address this question. Specifically, adeno-associated virus carrying ChR2 (a light-activated cation channel) or eNpHR3.0 (a light-driven chloride pump) were microinjected into the RN or other brain regions of Vglut2-IRES-cre mice or Vgat-IRES-cre mice to selectively activate or inactivate GLU or GABA neurons. We found that, 1) activation of RN GLU neurons significantly inhibited, while inactivation of RN GLU neurons potentiated cocaine self-administration (SA). In contrast, activation or inactivation of RN GABA neurons had no effect on cocaine SA; 2) activation of GLU neurons in the vHipp also failed to alter cocaine SA, which may be related to the very low density of vGluT2 expression in vHipp; 3) activation of RN GLU neurons altered neither basal nor cocaine-enhanced locomotion; and 4)

inactivation of RN GLU neurons supported self-administration of the intracranial inactivating laser light beam directed into the RN, while activation of RN GLU neurons failed to do so. Taken together, these findings, for the first time, suggest that RN GLU neurons play an important role in controlling cocaine SA and incentive motivational behavior. The precise mechanisms or neural circuitries underlying this action are unclear. The possible involvement of the RN-VTA and/or RN-NAc GLU projections is under intensive investigation.

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411.13/L13. ***Withdrawal from binge cocaine increases negative urgency in rats***

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Negative urgency, an important component of impulsivity, is defined as a tendency to engage in rash behavior when in a negative emotional state. Because negative urgency is a risk factor for developing substance abuse disorders and can help identify high-risk individuals early in life, outlining the relationship between negative urgency and exposure to drugs of abuse is important. Negative urgency is associated with cocaine dependence, and involves brain structures altered by exposure to cocaine. Here, we tested how previous binge cocaine consumption affects adult, male, Sprague-Dawley rats in a reward omission task. Because cocaine interacts with multiple transporters we also used 2 $\beta$ -carbophenoxy-3 $\beta$ -(4-chlorophenyl)tropane (RTI-113), a highly selective dopamine transporter (DAT) inhibitor, in an additional group of rats. Rats received daily injections (ip) for 7 d of either saline, cocaine (10-20 mg/kg), cocaine (20-40 mg/kg), or RTI-113 (3.0 mg/kg). To simulate an escalating dosage schedule seen in human binge consumption, the cocaine dose was increased linearly each day from the lower dose to the higher. Animals were then given a 15 d withdrawal period prior to training on the reward omission task. The task consisted of a free sucrose pellet with a light + tone followed by presentation of an active lever which gave additional pellets on an FR10 schedule. An inactive lever was also present. Reward omissions occurred on random 33% of the trials over total of 10 d; there was an additional 10 d of all-rewarded trials separating these omission days. Our results indicate that previous exposure to binge cocaine elevates levels of negative urgency in a dose-dependent manner on different behavioral dimensions. The lower dose of binge cocaine resulted in decreased reaction time on omission trials, but did not affect number of lever presses. Conversely, the higher dose of binge cocaine increased the number of lever presses per trial without affecting reaction time. The RTI-113 rats displayed a marked decrease in lever pressing. In sum, our findings demonstrate that previous binge cocaine consumption enhances discrete markers of negative urgency in rats. The results with RTI-113 indicate this effect is not mediated by DAT alone, and likely involves multiple mechanisms. Furthermore, our data add to the concern of a positive feedback cycle of substance abuse whereby personality risk variables are enhanced by exposure to the drug.

411.15/L15. ***Basal forebrain cholinergic lesions attenuate the reinstatement of cocaine-seeking produced by discriminative stimulus in goal-trackers but not sign-trackers***

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Goal-trackers (GTs), compared to sign-trackers (STs), express higher levels of acetylcholine when performing a cue detection and processing task. We hypothesized, therefore, that GTs utilize their basal forebrain cholinergic systems differently and to a greater extent than STs, such that this system may be critical for signal-induced behavior in GTs but not STs. The purpose of this experiment was to investigate individual variation in the reinstatement of drug-seeking behavior produced by a signal indicating cocaine availability (a discriminative stimulus), as well as the influence of the basal forebrain cholinergic system. STs and GTs were trained to self-administer cocaine using an intermittent access (IntA) procedure. The IntA procedure involved allowing animals access to cocaine for discrete 5-min drug available periods indicated by a light signal (DS+) separated by 25-min no drug available periods indicated by a different signal (DS-) in a different location than the DS+. This procedure results in 'spiking' brain levels of cocaine. Once stable performance was achieved on this procedure, animals underwent extinction training where the context remained similar to the IntA procedure but was now devoid of both DSs and an active response no longer had any consequence. STs and GTs did not differ in the acquisition or expression of self-administration or extinction training. After behavior was stably extinguished, half of the subjects received bilateral infusions of the cholinotoxic immunotoxin 19 IgG-saporin into the basal forebrain, while the other half received sham surgeries. Animals underwent 5 days of re-extinction. Finally, they underwent a reinstatement test during which the DS+ was presented non-contingently for 2 sec on a variable time schedule. Current results show that the ST-lesion group and both sham groups reinstated responding upon exposure to the DS+, compared to the last day of extinction. In contrast, the GT-lesion group did not reinstate responding, relative to the last day of extinction and, additionally, showed fewer active responses during the reinstatement test than the GT-sham group. Our findings suggest that the basal forebrain cholinergic system is involved in the reinstatement of drug-seeking behavior produced by a signal indicating drug availability in some animals (GTs), but not others (STs), further supporting the notion that drug cues are processed very differently in STs and GTs.

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411.16/L16. ***Falling for drug cues versus staying on task***

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Subjects vulnerable for addiction-like behavior have a propensity for transforming conditioned reward cues to cues that capture their attention and instigate cue-directed behavior. The goals of this research

are to develop a behavioral paradigm for determining the power of drug cues to shift subjects from performing a food-rewarded attention task to taking drug, to determine individual variation in the power of drug cues to do so, and to find treatments that increase the resistance of subjects to disengage from the task. Rats acquired an operant sustained attention task (SAT), which involved the reporting of hits and correct rejections (water-rewarded), and misses and false alarms (not rewarded) via retractable levers. Next, they were trained to self-administer cocaine, and after the acquisition of stable self-administration behavior, were shifted to a second self-administration procedure: intermittent access (IntA). For IntA training, cocaine access was limited to 5-min periods cued by a tone (DS+), alternating with 25-min timeout periods (DS-, white noise). After stable behavior, the propensity to shift from one task (SAT) to self-administration was assessed in chambers equipped with nose-poke ports placed on the opposite wall of the SAT intelligence panel. Animals were tested in one of three dual task situations: (i) SAT active nose poke had no consequence; (ii) SAT active nose poke during DS+ presentation received cocaine; (iii) SAT + active nose poke had no consequence but cocaine was passively administered during DS+ presentation. In IntA rats, the presentation of the DS+ at the 1 min mark evoked an immediate switch away from SAT to drug-taking when an active nose poke resulted in cocaine delivery in both STs and GTs. The effect of DS+ was amplified when presented at 40 min as animals completely disregarded the attention task. Ongoing research is testing whether passive, in contrast to self-administered, cocaine infusion during DS+ will also result in a switch in task. To this point, it does not appear that rats classified as sign- and goal-trackers differ in their vulnerability to DS+-evoked task shifts. Our paradigm models the power of drug cues, as opposed to unrestricted access to drug-taking, to deprioritize SAT performance and thus, is suitable for studying the cognitive and neurobiological mechanisms via which drug cues trigger drug-taking and relapse.

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411.17/L17. ***Incentive sensitization as a function of increasing experience with self-administered cocaine using an 'Intermittent Access' procedure***

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The temporal pattern of drug self-administration may be a very important determinant of subsequent changes in brain and behavior, but this variable has received relatively little attention. There has been considerable research using a "Long Access" model of self-administration that allows animals to self-administer drugs continuously for long periods of time (6 hours), thus maintaining sustained brain concentrations of drug. However, this model may not accurately reflect temporal patterns of human drug use, which can be very intermittent, leading to frequent 'spiking' brain concentrations of drug. The importance of the temporal dynamics of self-administration have been highlighted in a series of recent studies showing that a history of Long Access self-administration causes decreased, tolerance-like effects on dopamine release; whereas a different procedure, Intermittent Access (IntA) causes increased, sensitization-like effects on dopamine release. To better understand how these temporal dynamics impact motivation to use drugs we trained rats to self-administer cocaine using the IntA

procedure. To this end, rats were trained to self-administer cocaine using a procedure that assured all rats had equal cocaine exposure. Following acquisition of self-administration, rats were then tested for their motivation to work for cocaine and their willingness to take cocaine in the face of adverse consequences using two variations of a behavioral economic procedure. Following these baseline tests, rats were given 36 IntA self-administration sessions. The IntA procedure consisted of alternating 5 minute blocks of cocaine access and 25 minute timeout blocks. This procedure produced successive “spikes” in brain cocaine concentrations. After every 12 sessions of IntA the rats received a ‘probe test’ using the behavioral economic procedure to determine the influence of IntA self-administration experience on motivation to take cocaine. Our results indicate that all rats showed pronounced incentive sensitization following prolonged exposure to self-administered cocaine using the IntA schedule. Specifically, we found that the motivation to self-administer cocaine in the face of adverse consequences increased early in training and then remained relatively stable on all subsequent tests, and motivation to self-administer cocaine in the face of increasing price (effort required) increased on each successive probe test. In conclusion, experience with cocaine self-administration using an IntA procedure leads to incentive sensitization, consistent with previous reports that IntA results in sensitization of dopamine release.

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#### 411.19/L19. ***Mechanisms and reversal of adolescent cocaine-induced habits***

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Adolescence is a period of vulnerability to the development of many psychiatric disorders, including substance dependence disorders that persist across the lifespan. Incubation of certain biological factors associated with addiction may play a causal role. We explored this hypothesis in the context of cocaine-induced stimulus-response habits, which are increasingly considered a causal factor in the development and maintenance of addiction. Here, adolescent or adult mice were exposed to cocaine, and then decision-making strategies were characterized. Mice with a history of subchronic cocaine exposure in adolescence, but not adulthood, developed stimulus-response habits at the expense of engaging in goal-directed decision-making strategies. In addition to these behavioral changes, orbitofrontal prefrontal cortex (oPFC) dendritic spines were eliminated and dendrites were simplified in adults with a history of adolescent cocaine exposure. Stimulus-response habit formation was recapitulated by site-specific infusions of an Abl-family kinase inhibitor STI-571, which destabilizes dendrite structure, into the oPFC. Conversely, fasudil, a Rho-kinase inhibitor that can induce dendrite elaboration, enhanced response-outcome learning and memory, thereby blocking cocaine-induced habits. Cocaine-induced habits could also be blocked by ifenprodil, an NR2B-selective N-methyl D-aspartate (NMDA) receptor antagonist. The therapeutic-like effects of ifenprodil were occluded by infusion of STI-571 into the oPFC, indicating the ifenprodil acts by stimulating Abl-dependent structural plasticity in the oPFC. Together, these findings suggest that adolescent cocaine exposure biases decision-making strategies towards stimulus-response

habits by altering cellular structure during adolescent development, and that strategies aimed at reversing these events may reinstate, or enhance, goal-directed action selection in individuals with a history of cocaine exposure.

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411.20/L20. ***Maladaptive behaviors resulting from decreased glutamate release from astrocytes: phenotyping system xc- knockout rat***

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Excitatory signaling is achieved by an elaborate network involving reuptake and release mechanisms expressed by both neurons and astrocytes. System xc- (Sxc-) is a non-vesicular glutamate release mechanism primarily expressed by astrocytes that has been shown to exert profound control over multiple aspects of synaptic transmission. Sxc- is expressed in brain regions linked to diverse behaviors such as the basolateral amygdala, bed nucleus of the stria terminalis (BNST), nucleus accumbens (NAc), and medial prefrontal cortex (mPFC). As such, its dysfunction may contribute to CNS diseases ranging from drug addiction to schizophrenia. To determine whether glutamate release from astrocytes is essential for complex behavioral phenotypes, we created transgenic rats lacking Sxc- activity by mutating the Slc7A11 gene using Zinc-Finger Nucleases (ZFN). Genotyping data revealed successful mutations of Slc7A11 in two lines of Sprague Dawley rats. The first line contains a 39 base pair deletion in exon 2 leading to a frameshift and anticipated truncation of the xCT protein. The second line has a deletion of 13 amino acids corresponding to the majority of the 3rd transmembrane domain. The deletion of Sxc- function was verified in each line by demonstrating the lack of active <sup>14</sup>C-cystine uptake into brain slices or cultured cells. Mutant system xc- (MSxc) rats displayed normal survival rates, growth patterns, and basal levels of activity. However, MSxc rats exhibited significant differences in multiple behavioral assays including elevated plus maze, attentional set shifting, cocaine self-administration/reinstatement, and social interaction. Because many of the behavioral deficits exhibited by MSxc rats may reflect cognitive inflexibility, we have begun to examine key cortical areas for cellular, molecular, and structural abnormalities that could underlie these diverse maladaptive behaviors. To date, we have detected reduced PV expression in the prefrontal cortex and increased PV expression in the sensorimotor cortex. This pattern would be consistent with cognitive inflexibility stemming from an imbalance between goal- and habit-driven behaviors.

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411.21/L21. ***PACAP and cocaine reinstatement: A neuropeptide expressed by corticostriatal neurons that regulates nucleus accumbens astrocytes***

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Drug addiction involves heightened relapse vulnerability arising from persistent drug-induced neuro-adaptations, including a) hypofrontality which is thought to reflect reduced firing of cortical afferents to the nucleus accumbens (NAcc) and b) altered glutamate homeostasis in NAcc that likely involves reduced glutamate release and uptake by astrocytes. An important question is whether these forms of pathological plasticity are functionally linked such that reduced corticostriatal firing may result in aberrant regulation of astrocytes in the NAcc. To begin to evaluate this possibility, we first determined whether neurons regulate system xc- (Sxc) activity, a mechanism of non-vesicular glutamate release by astrocytes. We found that the rate of Sxc activity in astrocyte cultures was significantly increased in cells exposed to neuronal conditioned media achieved using neuronal inserts. These experiments demonstrate that releasable neuronal factors significantly upregulate Sxc activity. We hypothesize that the pituitary adenyl cyclase activating peptide (PACAP) may be the neuronal factor regulating glutamate release by astrocytes involving Sxc. First we determined that PACAP mimics a neuronal insert in that it significantly upregulates Sxc activity in astrocytes. Next, we verified the expression of PACAP in neurons from the prefrontal cortex (PFC) projecting to NAcc. Together, these data support the hypothesis that reduced corticostriatal firing may result in decreased PACAP release in NAcc which could potentially blunt Sxc activity in NAcc astrocytes. To determine whether this would impact relapse vulnerability, we microinjected PACAP into the NAcc and found that this significantly reduced cocaine-primed reinstatement, suggesting that increased PACAP signaling, consistent with other approaches capable of increasing Sxc activity, may blunt relapse vulnerability. In order to determine whether reduced PACAP signaling is sufficient to increase relapse vulnerability, we microinjected the PAC1R inhibitor PACAP6-38 into the NAcc. Preliminary data indicate that this is sufficient to produce an increase in cocaine reinstatement. Collectively, these studies demonstrate that neuropeptide PACAP is a powerful regulator of cocaine-related behaviors, likely through the modulation of glutamate homeostasis as maintained by astrocytes. As a result, an unrecognized consequence of hypofrontality may be impairing neuron-astrocyte interactions in a manner that determines the magnitude of relapse vulnerability.

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411.22/L22. ***Conditioned dopamine release scales with cocaine dose during Pavlovian conditioning***

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Phasic activity of midbrain dopamine (DA) neurons is thought to encode reward learning in the form of reward prediction errors (RPEs). RPEs reflect the difference between the expected and actual reward value: phasic DA activity increases when reward is better than expected and decreases when reward is worse than expected. In agreement with changes in firing rate, cue-evoked phasic DA release in the ventral striatum also tracks reward magnitude. The majority of studies demonstrating this finding, however, have investigated natural rewards (e.g., sucrose); whether or not cue-evoked phasic DA release also tracks the magnitude of drug reward has been unknown. Here we utilized fast-scan cyclic voltammetry during Pavlovian conditioning to test the hypothesis that cue-evoked phasic DA release

scales with the magnitude of cocaine reward. Rats were chronically implanted with microelectrodes bilaterally in ventral striatum and intravenous (i.v.) jugular catheters. Following recovery, rats underwent 20 Pavlovian conditioning sessions in which presentation of a light and lever was paired with delivery of cocaine; each session consisted of 10 trials. For the first 10 sessions in all rats, cues were paired with i.v. delivery of 0.4 mg/kg cocaine. Then, for the next 10 sessions, one group of rats continued to receive 0.4 mg/kg i.v. cocaine (n = 4), whereas the cocaine dose was increased to 0.8 mg/kg i.v. for the other group (n = 5). Cue-evoked phasic DA release increased significantly over the first 10 sessions in all rats. Over the next 10 sessions, however, cue-evoked phasic DA release decreased by 59% in the continued 0.4 mg/kg cocaine group, but increased by 346% in the 0.8 mg/kg group. Interestingly, cue-evoked phasic DA release did not differ between the 0.4 and 0.8 mg/kg groups until the end of testing (session 20). This study provides the first evidence that cue-evoked phasic DA release also tracks the magnitude of cocaine reward and demonstrates that cue-evoked ventral striatal phasic DA release can be “reengaged” by increasing the cocaine dose.

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411.23/L23. ***Roles of suvorexant, a dual orexin receptor antagonist, on impulsivity and cocaine-induced attention deficits***

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Lateral hypothalamic orexins (hypocretins) have functional roles in arousal, reward processing, attention and impulsivity. Concordantly, orexins project to neural substrates governing motivated behavior, including the locus coeruleus and ventral tegmental area, and orexins influence post-synaptic membrane activity through binding to orexin-1 and orexin-2 G-protein coupled receptors. Multiple psychopathologies, such as attention deficit hyperactivity disorder (ADHD) and substance abuse, are characterized in part by deficits in sustained attention and by impulsivity, but the role of orexins in mediating attention-related behaviors remains poorly understood. In the present study, we use behavioral pharmacology to investigate the effects of suvorexant, a dual orexin receptor antagonist approved by the Food and Drug Administration for treating insomnia, on impulsive behavior in rats using the five-choice serial reaction time task (5-CSRTT). Further, we examine the effects of suvorexant on cocaine-induced impulsivity. Lastly, we examine effects of suvorexant on orexinergic cell activity in a separate cohort using immunofluorescence targeting orexin-A and cFos antigens. Results demonstrate that suvorexant reduced the number of vehicle- and cocaine-elicited premature responses in 5-CSRTT, and that the reduction in cocaine-induced premature responses was not due to non-specific locomotor effects as cocaine elicited comparable hyper-locomotor effects in vehicle- and suvorexant-treated animals. Further, suvorexant increased orexinergic cFos-immunoreactivity within the lateral hypothalamus. These studies suggest potential therapeutic efficacy of orexin antagonism in reducing both non-induced and cocaine-elicited impulsive behaviors. Future studies should (i) elucidate and phenotype orexinergic circuits that underlie impulsive behaviors, (ii) examine effects of suvorexant on

sustained attention and cocaine self-administration and (iii) explore the mechanisms in which orexin governs impulsive behaviors.

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411.25/L25. ***Distribution analysis of intervals between cue-induced presses in rats trained to self-administer cocaine***

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Stimuli preceding drug self-administration sessions, the context of the experimental box and special signals accompanying intravenous drug injections are capable of inducing lever-pressing behavior after withdrawal from the drug. This phenomenon is known as cue-induced reinstatement of a response and has been investigated in hundreds of studies. However, inter-response times (IRTs) between cue-induced presses were analyzed only in one study. In the present study rats were trained to self-administer cocaine under fixed ratio FR1 schedule. After the first 16-17 training sessions of self-administration, every session started with phase of sham injections after placing animals into the experimental chambers when presses on the active lever were followed by light signals without cocaine injections. 85% of all presses were on the active lever. This phase of cue-induced presses continued until 3 min elapsed after the last active lever press. Then rats received cocaine priming injections and self-administration phase of the session started. Survival functions of the latencies to the first and to the last press were not significantly different from mono-exponential and bi-exponential function, respectively. IRTs between both active and inactive lever presses were distributed bi-exponentially. Distributions of the latencies to the first press or to the last press and distributions of IRTs were not significantly different from log-normal. IRTs increased both within and between sessions. The total number of presses decreased from session to session. The results suggest that regardless of continuing association between lever-pressing behavior and cues followed by cocaine administration in the second phase of the experiment, cue-induced presses demonstrated slow extinction not only after beginning of sessions but also from session to session. The bi-exponential distribution of IRTs suggests that there are two mechanisms controlling lever-pressing activity induced by cues associated with intravenous cocaine injections in rats.

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412.01/L26. ***Methamphetamine self-administration and foot-shocks produced differential molecular neuroadaptations in the hippocampi of punishment-sensitive and punishment-resistant rats***

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One of the main problems in the treatment of methamphetamine addiction is a high rate of relapse to drug use during abstinence. Methamphetamine-induced neuroadaptations in the brain are thought to play an important role in drug-seeking and relapse. Here we investigated the expression of neurotransmitter receptors in the hippocampus after punishment-induced and forced abstinence from methamphetamine self-administration in rats. We also measured the expression histone diacetylases (HDACs) that are involved in transcriptional regulation. We trained rats to self-administer methamphetamine (0.1 mg/kg/ injection) for 9 h/day for 20 days. Subsequently, for one group of rats, 50% of the lever-presses were punished by mild foot-shock (0.18 ->0.36 mA, 0.5 sec) for 1 days, while, for another group, lever-presses were not punished. control group of rats self-administered saline. Approximately 55% of punished animals reduced methamphetamine self-administration to about 20% of pre-shock level (punishment-sensitive rats), whereas the remaining animals continued to self-administer the drug (>50% of pre-shock level) in spite of electric foot-shocks (punishment-resistant rats). We euthanized rats at 21 days after 10-day punishment phase and extracted hippocampi for further analysis. We found decreases in dopamine D1 receptor (DRD1), HDAC9 and HDAC10 mRNA and protein levels in the hippocampus of punishment-sensitive animals in comparison to control and punishment-resistant groups. Our findings suggest that DRD1, HDAC9 and HDAC10 may be important substrates for the maintenance of or for relapse to methamphetamine self-administration. Studies are underway to identify the causal role that these molecular changes play in maintenance or suppression of methamphetamine self-administration.

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412.02/L27. ***Adolescent stress exposure increases vulnerability to addiction: Role of glutamate trafficking***

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Adolescence marks a key period of psychological and physiological development during which stressful experiences are poised to have dramatic impact on future behavior. As such, adolescence marks key period for vulnerability to current and future substance abuse. In particular, stress during adolescence, including low socioeconomic status, social isolation and chronic adverse life events, increases vulnerability to addiction. However, the underlying neurobiological mechanisms behind this vulnerability remain elusive. Therefore, the current studies utilized two adolescent stress paradigms in mice to determine their ability to modulate cocaine self-administration, extinction and reinstatement behavior. The first of these stressors was social isolation stress in which mice were isolated at weaning, preventing adolescent social play behavior. While this stress exposure did not alter the acquisition of operant learning or fixed ratio cocaine self-administration during adulthood, isolated animals exhibited greater motivation for cocaine, as evidenced by an increase in progressive ratio breakpoint. Furthermore, there is a trend towards an increase in cocaine seeking during cue-induced reinstatement. Studies are underway examining the effects of the second adolescent stressor, chronic unpredictable stress, on measures of cocaine taking and seeking during adulthood. Furthermore,

additional studies suggest that mouse with alteration in glutamate receptor trafficking exhibits enhanced response to stress exposure following cocaine. Studies examining the influence of adolescent stress on cocaine taking and seeking are underway in these mice. Funding was provided by NIH grant DA033372.

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412.03/L28. ***Effects of vaped delivery of psychomotor stimulants to rats***

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Abuse of, and addiction to, methamphetamine (MA), cocaine and, increasingly the cathinone derivative stimulants (“bath salts”, “plant food”, “flakka”), interferes with many aspects of personal health, vocational performance, interpersonal relationships and financial well being. Behavioral consequences of stimulant addiction also strain legal and emergency medical resources throughout the US. Stimulant abuse is therefore significant and continuing public health concern. Despite higher rates of treatment admissions for smoked versus other routes of cocaine administration and increasing proportions of MA users who smoke, there are no convenient and well developed models of intrapulmonary stimulant exposure available for rodent research. Studies were conducted in male rats to validate a novel drug delivery method using e-cigarette technology. Experiments demonstrated that vaped delivery of MA, 3,4-methylenedioxypyrovalerone (MDPV) or mephedrone for 40 minutes increased locomotor activity and decreased brain reward thresholds. Locomotor stimulation was similar to the effect of i.p. injection with 0.5-1.0 mg/kg of MA or MDPV and of 5.0 mg/kg mephedrone. The magnitude of effect on brain reward was comparable to i.p. administration of 0.5 mg/kg of MA or MDPV and of 1.0 mg/kg of mephedrone. Finally, it was found that rats will make nose-poke responses to obtain 2 min deliveries of MA or MDPV vapor in a self-administration paradigm. Thus, e-cigarette technology is effective for the intrapulmonary delivery of psychomotor stimulants to rats and results in physiologically relevant levels of drug exposure.

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412.04/L29. ***Chronic exposure to conditions of uncertainty promotes the pursuit of amphetamine***

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As with pathological drug use, pathological gambling (PG) is associated with symptoms of compulsion, loss of control and continuance of behavior despite negative consequences. Pathological gamblers are also at increased risk for developing substance use disorders suggesting that PG and drug addiction share common neuronal substrates. A defining feature of games of chance is uncertainty of outcome, a characteristic known to induce long-lasting neuronal alterations similar to those produced by repeated

intermittent exposure to stimulant drugs. Chronic exposure to uncertainty or intermittent exposure to stimulants both enhance subsequent locomotor and dopaminergic responding to amphetamine. Prior drug exposure is also known to increase predisposition to self-administer the drug. Here we show that chronic exposure to conditions of uncertainty also promotes the pursuit and self-administration of the stimulant amphetamine. Drug-naïve rats were trained to nose poke for a saccharin solution under predictable [fixed-ratio (FR)] or uncertain conditions [variable-ratio schedules (VR)] for up to 55 1-hr sessions given twice/day 6-days/week. During exposure, FR and VR rats did not differ in their saccharin intake, nose pokes emitted, or saccharin dipper entries. However, when given the opportunity to self-administer amphetamine (100 µg/kg/infusion, IV) two weeks later on progressive ratio schedule, rats previously exposed to uncertain saccharin reinforcement displayed enhanced work output and self-administration of significantly more drug infusions than rats exposed to conditions of predictable reinforcement. Taken together, these results indicate that chronic exposure to conditions of uncertainty can trigger neuroadaptations similar to those produced by repeated intermittent exposure to abused drugs that promote their enhanced pursuit and self-administration. Supported by NIH grant R01 DA 034184. Keywords: gambling, uncertainty, amphetamine, sensitization, locomotor, dopamine, self-administration, addiction

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**412.09/L34. *Effects of a histone deacetylase inhibitor on the induction of one-trial methamphetamine- and cocaine-induced behavioral sensitization in preweanling rats***

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The neural mechanisms mediating behavioral sensitization appear to differ depending on the length of the drug pretreatment regimen. As an example, the roles played by the D1 and D2 receptor systems vary when assessed using a one- vs. a multi-trial experimental procedure. Recently, epigenetic modifications have been recognized as important regulators of drug-induced plasticity. In terms of behavioral sensitization, the histone deacetylase inhibitor valproic acid was found to potentiate the multi-trial amphetamine-induced sensitized responding of adult mice. In contrast, valproic acid blocked the morphine-induced behavioral sensitization of adult mice when a one-trial procedure was employed. It is uncertain whether the differential actions of valproic acid (i.e., potentiating vs. blocking sensitization) are consequence of the single- vs. multi-trial experimental procedures or the different sensitizing agents that were used (amphetamine vs. morphine). The purpose of the present study was to determine whether pretreatment with valproic acid would potentiate or attenuate the one-trial methamphetamine (METH) and cocaine (COC) induced behavioral sensitization of preweanling rats. A developmental animal model was employed since preweanling rats show robust METH and COC one-trial behavioral sensitization, and preweanling rats have translational relevance for human models of drug addiction. In Experiment 1, preweanling rats (PD 16) were injected with saline or valproic acid (50, 100, 150, or 200 mg/kg) 15 min before single injection of saline or METH (4 mg/kg). One day later (i.e., on PD 17), rats

were challenged with METH (2 mg/kg) and locomotor sensitization was assessed across a 120 min testing session. In Experiment 2, preweanling rats were treated as just described, with the exception that COC (pretreatment, 30 mg/kg; test day, 20 mg/kg) was substituted for METH, and pretreatment and testing occurred on PD 19-PD 2 (preweanling rats exhibit stronger cocaine-induced behavioral sensitization at this age). Results showed that METH and COC caused robust one-trial behavioral sensitization in preweanling rats. Valproic acid neither affected basal locomotor activity on the pretreatment day nor altered the sensitized responding of METH- or COC-pretreated rats. Therefore, the present study suggests that histone hyperacetylation is not necessary for the one-trial psychostimulant-induced behavioral sensitization of preweanling rats. The different patterns of responding by young and adult animals may reflect ontogenetic differences in the neural mechanisms underlying behavioral sensitization.

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412.10/L35. ***One-trial and multi-trial methamphetamine-induced behavioral sensitization in preweanling rats: role of D2 receptors***

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In rodents, accumulating evidence indicates that the underlying neural mechanisms mediating one-trial and multi-trial behavioral sensitization differ. For example, the D1 receptor antagonist SCH23390 blocks the induction of one-trial cocaine-induced behavioral sensitization in adult rats, while not affecting the sensitized responding of adult rats given multiple cocaine administrations. The purpose of the present study was to determine whether pretreatment with a D2 receptor antagonist would differentially affect the one-trial and multi-trial methamphetamine (METH) induced behavioral sensitization of preweanling rats. A developmental animal model was employed since preweanling rats show robust one-trial behavioral sensitization, and we previously reported that one-trial cocaine-induced behavioral sensitization was not affected by D1 antagonist treatment. In Experiment 1 (one-trial sensitization), preweanling rats (PD 16) were injected with saline or raclopride (0.1, 0.5, or 1 mg/kg) 15 min before a single injection of METH (4 mg/kg). In a separate sub-experiment, rats were given either saline or a combined administration of SCH23390 (0.5 mg/kg) and raclopride (0.5 mg/kg) before METH treatment. Rats in the acute control group were given two injections of saline. After the second injection, rats were placed in activity chambers and distance traveled was measured for 30 min. One day later (i.e., on PD 17), rats were challenged with METH (2 mg/kg) and locomotor sensitization was assessed across 120 min testing session. In Experiment 2 (multi-trial sensitization), preweanling rats were treated as just described, with the exception that the pretreatment phase was conducted over four consecutive days (PD 13-PD 16), with testing occurring on PD 17. Results showed that neither raclopride nor SCH23390 + raclopride blocked the one-trial METH-induced behavioral sensitization of preweanling rats. In contrast, raclopride significantly attenuated the multi-trial behavioral sensitization of preweanling rats, with combined administration of SCH23390 + raclopride causing a greater disruption of sensitized responding than raclopride alone. These results provide further evidence that the neural mechanisms mediating

one-trial and multi-trial behavioral sensitization differ. Other disparities between one- and multi-trial behavioral sensitization have been reported; not least of which is the finding that contextual conditioning does not influence the one-trial behavioral sensitization of preweanling rats, while drug-environment associations strengthen the multi-trial sensitization of preweanling rats and adults.

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412.12/L37. ***Methamphetamine self-administration increases the expression of neurotrophic factors in sub-population of rats that show foot-shock-induced abstinence from methamphetamine***

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The transition from occasional to persistent drug use is thought to be associated with neuroplastic changes within the dorsal striatum, a key structure involved in habitual learning. Previous work in our laboratory has shown that methamphetamine (METH) self-administration (SA) in rodents is accompanied by changes in the expression of neuroplasticity-related genes in this brain region. Here we examined the influence of punishment on METH SA on the expression of genes that might distinguish between animals that continue to take the drug or refrain from doing so in response to repeated foot-shocks. Male Sprague-Dawley rats self-administered METH (0.1 mg/kg/injection, i.v.) or saline during twenty-two 9-h sessions. Following training, rats were subjected to incremental foot-shocks for thirteen additional sessions. After the foot-shock sessions, cue-induced drug craving was assessed at 2 and 21 days post-shock. Animals were euthanized 9 days after the second extinction test. Changes in gene expression were assessed using RT profiler-arrays and quantitative PCR (qPCR). Foot-shock punishment caused the separation of two distinct SA groups that consisted of rats that continued to seek METH despite the negative consequence (shock-resistant, SR) and rats that stopped METH seeking behavior (shock-sensitive, SS). During the extinction test, both SR and SS rats showed significantly higher cue-induced reward seeking on withdrawal day 21 relative to day 2. However, SR rats showed greater cue-induced drug craving during the relapse test compared to SS rats. There were significant differences in the expression of several genes in the dorsal striata of the two phenotypes. These genes included BDNF, VGF, Gfra1, Gfra2, NGF, and Ngfr, with SS rats showing higher expression than the SR rats. These findings suggest that neurotrophic factors within the striatum may act to implement plasticity-related changes that may promote some degree of protection against relapse in animals that respond to adverse consequences by stopping drug self-administration. These data provide further evidence that this model of adverse consequences better mimics human situations which may provide greater insight into the mechanisms of abstinence and relapses in clinical situations.

412.13/L38. ***Role of central nucleus of amygdala in cue-induced relapse to methamphetamine seeking after voluntary abstinence***

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Background: We recently established a new animal model of cue-induced methamphetamine seeking after prolonged voluntary abstinence (Caprioli et al. Biol Psychiatry 2015). Here, we studied the role of central amygdala (CeA) in this form of relapse. Methods: We trained rats to self-administer palatable food (6 sessions, 2-h/day) and then methamphetamine (15 sessions, 2-h/day). Next, voluntary abstinence (14 sessions) was achieved via discrete choice procedure between methamphetamine and palatable food (20 trials/day). We then assessed cue-induced methamphetamine seeking in extinction tests. We first determined the effect of systemic injections of the dopamine D1-receptor antagonist SCH39166 on cue-induced methamphetamine seeking and Fos expression in central amygdala (CeA) and areas that project to CeA: basolateral amygdala (BLA), anterior insular cortex (AIC), paraventricular thalamus (PVT), and ventral subiculum (vSub). Next, we determined the effect of CeA and BLA injections of SCH39166 on cue-induced methamphetamine seeking. Finally, we combined the retrograde tracer cholera toxin subunit-B (CTb, injected into CeA) with Fos to determine neuronal activation in the projection areas. Results: Cue-induced methamphetamine seeking after voluntary abstinence increased Fos expression in CeA, AIC, and PVT, but not in BLA and vSub; both Fos expression and drug seeking were decreased by systemic SCH39166 injections. CeA, but not BLA, SCH39166 injections decreased cue-induced methamphetamine seeking. Double-labeling analysis of CTb+Fos showed that cue-induced methamphetamine-seeking was associated with selective activation of AIC neurons that project to CeA. Conclusions: Results demonstrate a critical role of CeA in cue-induced relapse of methamphetamine seeking after voluntary abstinence and suggest a role of AIC projections to CeA in this relapse.

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412.14/L39.  ***$\beta$ -lactam antibiotic affects amphetamine-induced reinstatement in differentially reared rats: Evidence for glutamate homeostasis***

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Rats reared in an enriched condition (EC) demonstrate a variety of altered behaviors, including self-administration of amphetamine (AMP), when compared to rats reared in isolated (IC) and standard conditions (SC). Rearing in an EC has been shown to alter glutamatergic synaptic function. The glutamate homeostasis hypothesis of addiction proposes that excessive synaptic and extracellular glutamate levels promote the transition to compulsive drug taking. Our lab has shown that metabotropic glutamate receptors (mGluRs) are differentially altered by rearing condition and change self-administration on FR

and PR schedules of reinforcement, indicating that glutamatergic synaptic function mediates self-administration of AMP. However, glial transporter (GLT1) also helps maintain optimal synaptic and extracellular glutamate levels. During reinstatement synaptic glutamate levels are increased and GLT1 export of excess glutamate is reduced. Ceftriaxone (CTX),  $\beta$ -lactam antibiotic, has been shown to specifically upregulate GLT1. To understand how rearing condition alters GLT1 and determine how GLT1 is implicated in AMP reinstatement, rats were randomly assigned to saline (SAL) or CTX treatment after 3 day rearing period in EC, IC or SC, conditions. Rats were then implanted with indwelling jugular catheters and allowed to self-administer AMP (0.1 mg/kg/infusion) on a FR1 schedule of reinforcement during 60-min sessions. After stable responding was achieved, rats were administered 10 daily injections of SAL or CTX (200 mg/kg) after AMP self-administration. Rats were then exposed to extinction where active lever presses no longer resulted in AMP delivery. CTX did not alter AMP self-administration or extinction. After extinction, rats were exposed to AMP-induced (0.25 mg/kg, ip) reinstatement and active lever presses were measured. Results indicate that rats reared in the IC and SC, conditions showed a significant increase in active lever presses during AMP-induced reinstatement and EC rats did not show a reinstatement effect. There was a significant interaction of rearing condition  $\times$  treatment, such that CTX treatment blocked AMP-induced reinstatement in SC and IC rats. AMP-induced reinstatement did not increase inactive lever pressing across any groups. Our results indicate that EC protects against AMP-induced reinstatement and that rearing environment alters extracellular glutamate, as evidenced by the blockade of reinstatement following repeated CTX treatment. In addition, excess extracellular glutamate promotes reinstatement and provides evidence for the glutamate homeostasis hypothesis of addiction.

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412.15/L40. ***The 5ht1b receptor agonist, cp 94253, modulates methamphetamine self-administration***

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We previously found that the selective 5-HT<sub>1B</sub> receptor (5-HT<sub>1BR</sub>) agonist, CP 94253 (CP), shifts the cocaine self-administration (SA) dose-response (DR) curve upward and to the left during initial training but produces a downward shift after a 3-week period of abstinence from cocaine. This study examined if CP produced an abstinence-dependent effect on SA of another psychostimulant, methamphetamine (Meth). Male Sprague-Dawley rats were trained to self-administer Meth (0.1 mg/kg) on fixed ratio 5 (FR5) schedule of reinforcement until reinforcement rates stabilized. In addition in one experiment, rats commenced within-session DR training with access to five doses (0.003, 0.01, 0.03, 0.1, & 0.3 mg/kg) available for 3 min each presented in ascending order with 1 min time out between doses. Training continued until drug-intake stabilized again. Rats were then tested both pre- and post-abstinence for effects of CP on meth SA on FR5 as described above (Exp. 1) or on progressive ratio schedule of meth (0.03 mg/kg) reinforcement. For each type of SA schedule, rats were tested twice at both time points,

receiving CP (5.6 mg/kg, s.c.) prior to one test and saline (1 mL/kg, s.c.) prior to the other test, with order of these treatments counterbalanced. The results showed that on both pre- and post-abstinence tests, rats exhibited the typical inverted U-shaped Meth SA DR curve with the highest number of infusions obtained at the 0.01 mg/kg dose regardless of CP pretreatment. Prior to abstinence CP reduced the number of Meth reinforcers obtained for the two highest doses on the descending limb of the DR curve. Post-abstinence, CP had similar effects, reducing total reinforcers obtained for the three highest doses on the descending limb. CP also reduced the number of Meth reinforcers obtained and breakpoints on the progressive ratio schedule compared to saline pretreatment at both time points. Thus, unlike the abstinence-dependent modulatory role of 5-HT1BRs on cocaine SA, this study found similar effects of CP both pre- and post-abstinence. These results suggest that 5-HT1BR agonists may differentially modulate cocaine and Meth SA initially, but that after a period of abstinence, the agonist inhibits the reinforcing effects of both psychostimulants. These findings are important for understanding the clinical efficacy of 5-HT1BR agonists as treatments for psychostimulant disorders.

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412.16/L41. ***Extended access to MDMA and substituted cathinone self-administration in rats***

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The recreational use of (+)3,4-methylenedioxymethamphetamine (aka MDMA; “Ecstasy”) and of substituted cathinones continues to be a major public health issue in the United States. Studies have shown that extended access to intravenous (i.v.) self-administration of stimulants, such as cocaine and methamphetamine, results in escalation of drug intake relative to shorter access; however, little is known about the impact of extended access on self-administration of entactogen class stimulants such as MDMA or the substituted cathinones methylone and 4-methylmethcathinone (4MMC; mephedrone). Male Wistar rats were randomly assigned to short-access (ShA, 2-hr) and long-access (LgA, 6-hr) groups and trained to self-administer MDMA, methylone or 4MMC (0.5 mg/kg/infusion), using fixed-ratio (FR) testing procedures. The groups were then evaluated on a progressive-ratio (PR) dose-substitution (0.01-2.5 mg/kg/infusion) procedure. Rats given LgA to methylone and 4MMC earned more infusions than rats trained with LgA MDMA during acquisition, however only rats assigned to 4MMC LgA exhibited escalation of self-administration during the initial 2-hr of sessions. PR test results demonstrated an increase in drug-intake and breakpoints in rats assigned to LgA training of 4MMC or methylone relative to rats assigned to ShA training; however, rats assigned to LgA training of MDMA failed to increase drug-intake relative to rats assigned to ShA training. These findings show that access duration differentially impacts the acquisition and maintenance of the self-administration of MDMA, methylone and 4MMC, and that the relative reinforcing value of MDMA and substituted cathinones in rat self-administration differs.

412.17/L42. ***The effects of varenicline on methamphetamine self-administration and drug-primed reinstatement in male and female rats***

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Long-term methamphetamine (meth) use can result in severe dental problems, malnutrition, damage to the cardiovascular system, memory loss, psychotic behavior, anxiety, confusion, insomnia, mood disturbances, and violent behavior. These negative health consequences make treatment of meth addiction a priority. Unfortunately, meth cessation remains challenging, with no approved medications for its treatment and a majority of addicts returning to use within 6 months of treatment. Research has revealed sex differences in meth addiction vulnerability. For example, women on average are more susceptible to meth dependence while starting to use meth and, once dependent, women are more difficult to treat and more prone to relapse. Preclinical animal models rarely use female subjects. This oversight leaves a critical need for basic animal research on meth-taking/seeking in females. With this background in mind, the goal of the present study was to examine the effects of varenicline, a partial  $\alpha 4\beta 2$  and full  $\alpha 7$  nicotinic acetylcholine receptor agonist, on meth self-administration and reinstatement. Further, we assessed the impact of this pharmacological intervention in male and female rats. Male and female Sprague-Dawley rats were surgically implanted with an indwelling jugular catheter. Half of the rats were then trained to self-administer 0.05 mg/inj meth (Meth-Male; Meth-Female); the other half self-administered saline (Sal-Male; Sal-Female) during daily 1-hour sessions. When responding stabilized, varenicline (0.0, 0.3, 1.0, 3.0 mg/kg) was tested to determine how varenicline altered meth taking. Varenicline was probed on 4 test days; each test separated by 2 standard self-administration sessions to assure responding remained stable. This testing was followed by 15 extinction sessions. Four consecutive days of meth-primed reinstatement followed 24 hours after the last extinction session. The same 4 doses of varenicline (0.0; 0.3; 1.0; 3.0 mg/kg) were examined in a randomized order for each rat to determine how varenicline altered reinstatement primed by 0.25 mg/kg meth (IP). Male and female rats readily self-administered meth. Varenicline dose-dependently reduced meth-taking in female, but not male rats. Meth-Female rats displayed more robust meth-primed reinstatement compared to all other groups. Interestingly, the lower doses of varenicline increased meth-primed reinstatement. Although varenicline decreased drug-taking in female rats, increased susceptibility to reinstatement (i.e., relapse) may be an impediment for the use of varenicline as a therapeutic to treat meth use disorder.

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412.18/L43. ***mouse model for binge methamphetamine use***

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Methamphetamine (MA) use in humans is often reported to involve repeated binges separated by intermittent periods of withdrawal. Animal models that show similar binge-like patterns of MA intake are missing. We have used selective breeding to produce mouse lines that consume higher (MAHDR) and lower (MALDR) amounts of MA in two-bottle choice (water vs. MA dissolved in water) drinking procedure. To create a more representative animal model of MA use in humans, the MAHDR mice are being studied to determine if procedural variations will result in binge-like patterns of MA drinking. Such model would be useful for medications development. We explored the impact of factors: (1) the concentration of MA in the two-bottle choice procedure; (2) the number of bottles containing MA vs. water, and (3) the length of the withdrawal period between MA drinking periods. In study 1, the impact of MA concentration and number of bottles was examined in mice that were given access to water vs. MA, with the concentration of MA increased every 4 days from 20 to 140 mg/L. Groups of mice were offered MA in 1, 2 or 3 bottles. For comparison, MALDR mice were also tested. The impact of withdrawal period was examined in DBA/2J inbred strain mice, a progenitor of the MAHDR line, and then in MAHDR mice. Thus, in study 2, DBA/2J mice were offered water vs. 3 bottles of MA at increasing concentrations from 20 to 80 mg/L, and then an intermittent withdrawal period of 30 h was initiated for comparison to previous data using a 6-h withdrawal period. Study 3 in MAHDR mice, used withdrawal periods of 6, 30 and 78 h. MAHDR mice escalated their MA intake with each increase in concentration of MA. They more than doubled their MA intake when 3 MA containing bottles were offered vs. water, as compared to when the number of MA and water bottles was the same. MAHDR mice escalated their consumption up to 30 mg/kg of MA in an 18-h period on average, which would be considered a binge-like level of consumption. MALDR mice consumed negligible amounts of MA, regardless of the procedure. DBA2J mice also consumed increasing amounts of MA with each increase in MA concentration. However, the intermittent 30-h MA withdrawals were associated with incremental reductions in MA intake in DBA2J mice, with a 3-fold decrease after 9 intermittent withdrawal sessions. In contrast, the 6-h and 30-h MA withdrawal sessions had no impact on the high binge-like MA intake of MAHDR mice, and the 78-h withdrawal session was associated with only a small suppression in MA intake. The MAHDR line is ideal for the development of genetic mouse model of binge-like MA consumption in which manipulations that may reduce high levels of MA intake can be studied.

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412.19/L44. ***Role of dopamine and adenosine receptor stimulation on methamphetamine seeking in rats***

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Methamphetamine abuse is a global health problem, however the neurobiology is not well understood. Previous work has shown that dopamine and adenosine receptors are involved in methamphetamine reinforcement. The following studies examined the role of dopamine and adenosine receptors in the

reinstatement to methamphetamine seeking, a rodent model of relapse. Rats were trained to lever press for methamphetamine in daily 2-hr self-administration sessions on a fixed-ratio 1 schedule for 10 consecutive days. After one day of abstinence, lever pressing was extinguished in 6 daily extinction sessions. We first assessed whether stimulation of dopamine D1 or D2 receptors is sufficient to induce methamphetamine seeking. Thus, SKF 81297 (D1 agonist) and quinpirole (D2 agonist) were administered prior to a reinstatement test session. Both SKF 81297 and quinpirole exhibited a dose-dependent increase in methamphetamine seeking, although SKF 81297 was substantially less effective at the peak dose. We then identified whether quinpirole-induced reinstatement would be altered by systemic administration of the A1 agonist, CPA, or the A2A agonist, CGS 21680. Quinpirole-induced reinstatement was blunted by pretreatment with either CPA (0.03 mg/kg) or CGS (0.1 mg/kg). Finally, systemic D1 agonist administration is known to blunt cocaine seeking. Therefore, we examined whether an analogous effect would occur with D1 agonist administration on methamphetamine seeking. Systemic administration of methamphetamine (1.0 mg/kg) produced robust reinstatement that was attenuated by pretreatment with SKF (1.0 mg/kg). Collectively, these data suggest that the dopamine and adenosine systems play an integral role in methamphetamine-seeking behavior.

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#### 412.20/M1. ***A rat model of social stress-potentiated methamphetamine seeking***

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Substance use disorder (SUD) is a chronic disorder characterized by long-term vulnerability to relapse, even after treatment. Following rehabilitation, patients encounter increased drug availability and stress, both of which trigger relapse and accidental overdose. Studies in humans show that social stressors trigger particularly strong drug craving; however, it is not known how social stress affects neuronal function to increase vulnerability to relapse. Animal models allow for the dissection of neural mechanisms at a level that cannot be explored in humans, but current animal models most often use physical stress to trigger relapse. Therefore, we have developed a rat model of social stress-potentiated methamphetamine (METH) seeking that more closely recapitulates the post-rehabilitation challenges of SUD patients. For the relapse model, male Sprague Dawley rats were trained to self-administer METH through jugular catheters. Following extinction, the rats were either exposed to social defeat stress by a male Long Evans retired breeder or placed in a clean cage for the same length of time. A reminder of the social defeat session did not reinstate lever pressing in defeated rats. However, when the social stress reminder was presented prior to a METH priming injection, defeated rats showed significantly potentiated lever pressing compared to non-defeated rats receiving a clean cage reminder prior to the METH priming injection. Furthermore, the combination of social stress and METH priming injection triggered lever pressing levels that were significantly higher than initial training levels. Thus, the degree of drug seeking is reminiscent of the strong motivation to drug seek that has been reported in SUD patients in the presence of social stress and drug availability. These results suggest that this model of social stress-potentiated METH seeking may be a useful tool for further investigation of the neural

mechanisms underlying relapse. Regional cellular activation is currently being assessed to identify key brain regions involved in the potentiation of METH seeking by social stress. Ultimately, these studies may be valuable for the development and screening of therapeutics that decrease vulnerability to relapse in SUD.

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**412.22/M3. *d-Methamphetamine self-administration in rats: Specific antagonism with blockade of the vesicular monoamine transporter***

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Reinforcing effects of amphetamines, substrates for the dopamine (DA) transporter (DAT), and cocaine, DAT inhibitor, have common capacity to increase extracellular DA levels in terminal regions of mesolimbic dopaminergic neurons. Amphetamines, after accumulation in cytoplasm via DAT, release DA through actions at the vesicular monoamine transporter (VMAT). Recent studies suggest that VMAT2 inhibitors can decrease methamphetamine self administration. In efforts to discover novel potent and selective ligands for VMAT2, a series of enantiomeric 10-substituted-tetrabenazine (TBZ) derivatives was designed, synthesized, and screened for displacement of radioligands bound to VMAT2 (labelled with [<sup>3</sup>H]dihydro-TBZ) and DAT ([<sup>3</sup>H]WIN 35,428). The compound [(+)-CYY477-1] most potent (K<sub>i</sub>=8.77 nM) at VMAT2 and selective (no displacement of [<sup>3</sup>H]WIN 35,428 up to 0.1 mM) was studied for its behavioral effects alone and in combination with d-methamphetamine or cocaine. Up to doses of 0.1 mg/kg (ip) (+)-CYY477-1 had no effects on locomotor activity in mice, and decreased activity at 1.0 mg/kg. (+)-CYY477-1 (0.3-10 mg/kg) dose-dependently blocked locomotor stimulant effects of d-methamphetamine (3 mg/kg, ip) in mice, though at doses above the dose that decreased activity when administered alone. However, those doses had no effect on the stimulation of activity produced by cocaine (20 mg/kg, ip). d-Methamphetamine, d-amphetamine (each: 0.032-0.32 mg/kg/inj, iv) and cocaine (0.1-1.0 mg/kg/inj, iv) were self administered above saline levels, whereas (+)-CYY477-1 was not, up to a dose of 0.032 mg/kg/inj. Pretreatment with (+)-CYY477-1 (0.001-0.01 mg/kg, ip) dose-dependently decreased the maximal self administration of d-methamphetamine and d-amphetamine but not cocaine. Further, the VMAT2 inhibitor, dihydro-TBZ (0.032 mg/kg, ip), also decreased maximal self administration of d-methamphetamine, however with less apparent potency than (+)-CYY477-1. Neither (+)-CYY477-1 nor dihydro-TBZ had effects on food-reinforced behavior at doses that decreased drug self administration. The DA uptake inhibitor, WIN 35,428 (0.1-1.0 mg/kg, ip), dose-dependently shifted the d-methamphetamine or cocaine dose-effect curves to the left. The present results suggest distinct effects of VMAT and DAT inhibitors on the reinforcing effects of substrates or DAT inhibitors. Further, the VMAT may serve as target for the development of treatments for amphetamine abuse. Supported by NIDA IRP (JLK) and grants NSC 101-2325-B-002-010, from National Science Council, Republic of China and 10R71614-1 from the National Taiwan University (LWH).

506.01/L34 ***Effect of neuron-specific deletion of Rbfox1 on learning and cocaine-related behaviors in mice***

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Rbfox1 (A2BP1; Fox1) is a member of the Rbfox family of splicing regulators that displays high levels of expression in brain. Molecular genetic studies in humans associate variants in Rbfox1 with vulnerability to substance dependence as well as epilepsy, schizophrenia and intellectual disability/ autism. Genes whose primary transcripts are differentially spliced in brain and/or differentiated stem cells include many that have been implicated in addiction and in glutamatergic neurotransmission. Previous studies have shown that the brains of CNS-specific Rbfox1 knockout mice were hyperexcitable and that the homozygous knockout mice display epileptiform activities. Here, we expand the behavioral characterization of these mice, adding novel data for heterozygous Rbfox1 mice that might more accurately model common human level- of- expression variation. Rbfox1 knockout mice exhibit impaired learning/memory, altered sensitivity to cocaine reward as assessed by conditioned place preference test and baseline and cocaine-induced locomotor activities that are indistinguishable from those in wildtype littermates. These behavioral observations are consistent with contributions of variants in this gene to human phenotypes that include vulnerability to substance use disorders. Support: NIH IRP (NIDA).

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506.05/L38. ***A within-animal assessment of neural ensembles associated with novelty and cocaine***

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Novelty seeking is a personality trait associated with an increased vulnerability for substance abuse. In rodents, elevated novelty seeking has been shown to be a predictor for elevated drug self-administration and compulsive use. While previous studies have shown that both novelty and drugs of abuse have actions within similar mesocorticolimbic regions, little is known as to whether the same neural ensembles are engaged by these two stimuli. In this project, we wanted to determine the activation patterns associated with novelty and cocaine. Using the TetTag mouse model (a dual transgenic reporter line that allows for long lasting temporally controlled tagging of active neurons), we compared neurons engaged by cocaine and novelty seeking. We investigated the infralimbic and prelimbic prefrontal cortex, the nucleus accumbens (NAc) core and shell, the ventral hippocampus, and the basolateral amygdala for overlap between neurons associated with cocaine and novelty seeking, and found significant overlap, especially in the NAc core. To test the functional significance of overlap between neural encoding of novelty and cocaine, we are using the TetDREADD mouse model; a variant of the TetTag mouse that yields activity-dependent expression of Gi/o coupled DREADD receptors

(hM4Di) in a temporally controlled manner. TetDREADD mice were trained to self-administer cocaine and novelty (operant sensation seeking: OSS) in different contexts to test the ability of silencing neurons engaged during one of these behaviors to affect expression of the other behavior. The data suggest that increasing Gi/o signaling in neurons engaged during cocaine self-administration can reduce OSS. Ongoing studies are further parsing the functional overlap of neurons involved in cocaine and novelty seeking.

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506.06/L39. ***Behavioral and physiological effects of novel kappa opioid receptor based DREADD in rats***

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In the past decade, novel methods using engineered receptors have enabled researchers to manipulate neuronal activity with increased spatial and temporal specificity. One widely used chemogenetic method in mice and rats is the DREADD (designer receptors exclusively activated by designer drugs) system in which a mutated muscarinic G-protein coupled receptor is activated by an otherwise inert synthetic ligand, clozapine-n-oxide (CNO). Recently, the Roth lab developed a novel inhibitory DREADD in which a mutated kappa opioid receptor (KORD) is activated by the pharmacologically inert drug salvinorin B (SalB; Vardy et al., 2015). They demonstrated the feasibility of using KORD to study brain circuits involved in motivated behavior in mice. Here we used behavioral, electrophysiological, and neuroanatomical methods to demonstrate the feasibility of using the novel KORD to study brain circuits involved in motivated behavior in rats. In Exp. 1, we show that SalB dose-dependently decreased spontaneous and cocaine-induced locomotor activity in rats expressing KORD in midbrain (ventral tegmental area/substantia nigra). In Exp. 2, we show that SalB completely inhibited tonic firing in KORD-expressing putative dopamine neurons in midbrain. In Exp. 3, we used a 'retro-DREADD' dual-virus approach to restrict expression of KORD in ventral subiculum neurons that project to nucleus accumbens shell. We show that KORD activation selectively decreased novel context-induced Fos expression in this projection. Our results indicate that the novel KORD is an excellent tool to selectively inactivate brain areas and neural circuits in rat studies of motivated behavior.

506.10/L43. ***Glycogen synthase kinase 3 in the rat ventral hippocampus is necessary for the development of cocaine-induced behavioral sensitization***

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The ventral hippocampus is involved in drug-seeking behavior and psychostimulant-induced behavioral sensitization. We have previously demonstrated that rats exposed to repeated cocaine exhibit greater increases in both locomotor activity and dopamine efflux in the medial shell of the nucleus accumbens in response to NMDA stimulation of the ventral hippocampus. Furthermore, cocaine-sensitized rats exhibit greater density of NR2B-containing NMDA receptors in the ventral hippocampus. Glycogen synthase kinase 3 (GSK3) is a significant mediator of many intracellular signaling pathways, including increases in NMDA receptor function and trafficking of NR2B-containing NMDA receptors to the cell surface. Therefore, the current study examined whether inhibition of GSK3 in the ventral hippocampus diminishes cocaine-induced locomotor activity and the development of locomotor sensitization. Male rats were bilaterally infused with vehicle or the selective GSK3 inhibitor SB216763 (1ng) into the ventral hippocampus, 20 min prior to cocaine (15 mg/kg, ip.) or saline (1ml/kg, ip.) once daily for five days. After ten day abstinent period all rats were challenged with cocaine injection in the absence of microinfusions. Pretreatment with the selective GSK3 inhibitor SB216763 into the ventral hippocampus significantly reduced cocaine-induced activity on days 2-5. Further, pretreatment with SB216763 attenuated the development of sensitization to the locomotor-stimulating effects of cocaine. The role of GSK3 in the regulation of NMDA receptors in the ventral hippocampus of cocaine-sensitized rats is currently under investigation. Overall, the findings suggest that GSK3 activity within the ventral hippocampus is necessary for the development of sensitization following repeated cocaine exposure. NMDA receptors may represent an important target for cocaine-activated GSK3 in the ventral hippocampus.

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506.12/M1. ***A role for nucleus accumbens somatostatin interneurons in cocaine induced plasticity***

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The nucleus accumbens (NAc) is a brain region that is involved in regulating behavioral responses to both natural and drug induced reward. While the NAc is mainly comprised of D1- or D2-receptor expressing medium spiny neurons (MSNs), there are several classes of GABAergic Interneurons in the area as well. MGE (medial ganglionic eminence)-derived somatostatin (Sst) expressing interneurons account for 2-3% of the total neurons in the region and have previously been shown to be activated

after cocaine administration. We show that after 7 days of I.P. cocaine Sst transcription is increased in the NAc up to 4 hours following the last injection. This supports our previously reported finding that 7 days of IP cocaine leads to a decrease in H3K27Me3 binding at the Sst promoter in NAc. We utilized optogenetics, viral mediated circuit tracing, along with stem cell transplantation of MGE interneuron precursors to study the role of NAc Sst interneuron populations in behavioral responses to cocaine. We found that NAc Sst interneurons are innervated by many of the same glutamatergic, dopaminergic, and serotonergic afferents as D1/D2 MSNs in the region and that these circuits undergo remodelling after chronic cocaine exposure. Using optogenetics in vivo to control the activity of NAc Sst interneurons, we show that stimulation of NAc Sst interneurons suppresses locomotor responses and conditioned place preference (CPP) to cocaine, whereas silencing the interneurons has similar but distinct effects, suggesting that the activity of NAc Sst-interneurons plays a critical role in regulating cocaine induced plasticity in the NAc. We next studied the effect of transplanted fetal MGE cells in cocaine action and found that MGE transplantation into NAc, which produces an increase in the number of Sst interneurons, reduced CPP scores, suggesting that transplanted Sst-interneurons are functionally equivalent to endogenous NAc Sst interneurons. Finally, we performed RNA-seq on FACS isolated NAc Sst interneurons after chronic cocaine administration in vivo and identified genome wide transcriptional changes induced by cocaine specifically in this cell type. By combining molecular and behavioral analyses of this specific cell type, we hope to contribute to the fields of addiction and stem cell research to ultimately gain a deeper understanding of the fundamental molecular, electrophysiological, and epigenetic mechanisms that regulate cocaine-induced neuroplasticity.

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506.13/M2. ***Cocaine regulates monoubiquitination of histones H2A and H2B in nucleus accumbens***

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Although the etiology of drug addiction is multi-factorial, mounting evidence suggests that drug-induced alterations in gene expression in the brain's reward circuitry contribute to the chronic, relapsing nature of the syndrome. Our group and others have shown that histone post-translational modifications represent one important mechanism by which chronic exposure to cocaine induces these changes. Histone ubiquitination, despite being known to exist for decades, is less well studied than other histone marks. The dominant form of ubiquitinated histones in the cell are monoubiquitinated H2A and H2B, and both have been shown to regulate transcription in yeast and cultured human cells. However, very little is known about the role of histone ubiquitination in brain, especially in disease models such as drug addiction. Here, we show that repeated cocaine administration in mice and cocaine self-administration in rats regulate levels of H2A and H2B monoubiquitin as well as levels of monoubiquitin 'writer' and 'eraser' enzymes in the nucleus accumbens, a key brain reward region. Quantitative ChIP is currently being performed to identify how these marks are distributed at genes known to be regulated by

cocaine. We are additionally examining the effect of histone monoubiquitin on behavioral responses to cocaine by viral-mediated overexpression or knockdown of H2A ubiquitin ligases Ring1 and RNF2. To our knowledge, this is the first evidence for a role of histone ubiquitination in the pathophysiology of drug addiction and points to a new area of research with potential therapeutic benefits.

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**506.14/M3. Cocaine augments local synaptic translation in the nucleus accumbens through small GTPase network**

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Dendritic spines are the sites of most excitatory synapses in the central nervous system, and withdrawal from drugs of abuse alters the density and morphology of dendritic spines on medium spiny neurons (MSNs) of the nucleus accumbens (NAc), a primary reward region. Members of the Rho subfamily of Ras-like small GTPases are critical regulators of spine morphogenesis in MSNs, and guanine nucleotide exchange factors (GEFs) directly activate small GTPases. Our studies indicate that early withdrawal from both investigator-administered and self-administered cocaine increases the synaptic expression of the Rap1 small GTPase in the NAc. Conversely, late withdrawal from cocaine decreases synaptic NAc Rap1 levels. To date, no downstream effectors of Rap1 in NAc MSNs have been identified, and here we characterize a novel role for Rap1 in stimulating the activity of an AKT/mammalian target of rapamycin (mTOR) local translation network within dendritic spines. Via viral-mediated gene transfer and pharmacological manipulations, we found that altered Rap1-AKT-mTOR signaling controls NAc spine morphogenesis with resulting time-dependent effects on cocaine-mediated behavioral reward. Using optogenetic methods we dissected the excitatory inputs to the NAc that regulate Rap1-AKT-mTOR signaling. These optogenetic studies revealed a specific role for prefrontal cortex (PFC) to NAc projections in increasing synaptic Rap1-AKT-mTOR activity, and we found that PFC terminal stimulation in the NAc increases behavioral reward through Rap1. Our recent work has identified specific proteins that are locally synthesized in NAc synaptosomal fractions through mTOR, and current studies are aimed at determining how these locally formed proteins regulate cocaine-mediated spine morphogenesis and behavioral reward. Supported by NIDA

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**506.15/M4. Cocaine-induced enhancement of D1, and suppression of D2, medium spiny neuron activity in the nucleus accumbens is associated with cocaine seeking**

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Reward learning is robust and long-lasting, and cue/context presentation elicits seeking for the previously paired reward. This process is dysregulated in cocaine addiction, whereby potentiated cue/context-reward associations combined with an inability to extinguish previously learned drug associations are thought to drive relapse. Previous work has defined the role of dopaminergic neurotransmission in cue/contextual learning and outlined how these processes are altered in nucleus accumbens (NAc) following cocaine administration; however, dopamine has opposing actions at D1- versus D2-type medium spiny neurons (MSNs) in the NAc, making it critical to determine how cocaine produces lasting alterations to each of these neuronal subpopulations. Utilizing fiber photometry calcium imaging in freely moving animals, we dissected the cell-type specific mechanisms encoding cocaine reward as well as context-elicited cocaine seeking. We virally targeted the genetically encoded calcium indicator, GCaMP6f, in mice that express Cre-recombinase in D1 or D2 MSNs and recorded activity in NAc during acquisition, expression, and extinction of conditioned place preference. Subsequently, we determined how prior chronic exposure to cocaine altered these processes. Consistent with previous work, acute cocaine administration increased D1 MSN activity, while reducing D2 activity, suggesting that biasing towards D1 MSN output is a critical determinant in the ability to form context associations. Further, we found that D1 and D2 MSNs encode very different temporally specific information about associations. Enhanced D1 MSN activity immediately preceded entry into the drug-paired context, while D2 activity was suppressed only after entry into this context. Both D1 and D2 effects diminished across extinction of place preference. Chronic cocaine administration facilitated place preference and impaired extinction, effects which were positively correlated with D1 activity, suggesting that cocaine experience strengthens context/cue-reward associations via augmented D1 firing. Together, we demonstrate distinct temporal patterns of D1 and D2 MSN signaling in the NAc associated with cocaine reward learning, providing new insight into the circuit basis of drug-cue associations and drug seeking. Supported by NIDA DA008227, the DARPA Neuro-FAST program, and the Gatsby Foundation

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506.16/M5. ***Activation of estrogen receptors in the nucleus accumbens enhances the development of cocaine conditioned place preference in female mice***

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Clinical studies indicate that there are sex differences in behavioral responses to cocaine, with females being more sensitive than males. Findings from humans and animals suggest that estradiol might contribute to these differences. The aim of this study was to identify the specific estrogen receptor (ER) that modulates the rewarding properties of cocaine and to investigate the molecular mechanisms responsible for this effect. Ovariectomized female mice were treated with specific agonists to ER $\alpha$  or

ER $\beta$  in the cocaine conditioned place preference (CPP) protocol. Mice underwent conditioning sessions (3 with intraperitoneal injections of 5 mg/kg cocaine and 3 with saline). ER agonists were administered 1 hour before each conditioning session. We found that the ER $\beta$  agonist, diarylpropionitrile (DPN; 1 and 5mg/kg) significantly increased cocaine CPP compared to a vehicle-treated group. Activation of ER $\alpha$  by propylpyrazoletriol (PPT; 1mg/kg) also resulted in a slight increase in cocaine CPP, but this increase was not statistically significant. To complement these findings, we stereotaxically injected lentiviral vectors that express short hairpin RNAs (shRNAs) targeting ER $\alpha$  or ER $\beta$  in the nucleus accumbens of intact female mice. Mice injected with lentivirus expressing either ER $\alpha$  or ER $\beta$  shRNAs exhibited decreased cocaine CPP compared to mice injected with lentivirus expressing a control non-targeting shRNA. Together, our results suggest that activation of both ER $\alpha$  and ER $\beta$  might play a role in the enhancement of cocaine reward in females.

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506.22/M11. ***GIRK channels in VTA DA neurons regulate the sensitivity of the mesolimbic DA system to cocaine***

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The ventral tegmental area (VTA) is a key anatomic substrate for reward, for both naturally reinforcing stimuli and drugs of abuse. The VTA is a heterogeneous nucleus consisting of dopamine (DA), GABA, and glutamate neurons. DA neurons are the most abundant neuron population in the VTA, and they mediate the increase in DA neurotransmission in the mesolimbic DA system triggered by in vivo exposure to drugs of abuse. As the loss of GIRK channel activity in projection targets of the VTA, such as the mPFC, are also known to show enhanced behavioral sensitization to cocaine, here we evaluated the impact of the loss of GIRK channels in VTA DA neurons on reward-related behaviors triggered by cocaine. Our preliminary data using a novel conditional mouse line where *Girk2* was ablated in DA neurons (DATCre:*Girk2*flox/flox mice) show that loss of GIRK2 blunts the GABABR-dependent inhibition of VTA DA neurons. In these studies we also show that GIRK2 ablation leads to loss of autoreceptor-dependent inhibition in VTA DA neurons. This correlates with enhanced locomotor responses to acute and repeated cocaine relative to control mice. Moreover, data from conditioned place preference and other reward-related assays in DATCre(+):*Girk2*flox/flox mice, along with regional and cell-type specific DREADD manipulations, suggest that GIRK channel activity in VTA DA neurons controls the sensitivity of the mesocorticolimbic system to cocaine.

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506.23/M12. ***Ablation of the patch compartment reduces cocaine-induced stereotypy***

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Repeated exposure to cocaine (COC) induces stereotypy, which is characterized as inflexible, repetitive behavior. Enhanced relative activation of the patch compartment of the striatum has been shown to positively correlate with the emergence of stereotypy following repeated COC treatment, suggesting that stereotypy may be related to preferential activation of this region. However, the specific contribution of the patch compartment to COC-induced stereotypy following repeated exposure is unknown. To elucidate the involvement of the patch compartment to the development of stereotypy in response to repeated COC exposure, we determined if destruction of this sub-region altered COC-induced behaviors. Animals were bilaterally infused in the striatum with the neurotoxin dermorphin-saporin (DERM-SAP; 1 ng/[[Unsupported Character - Symbol Font &#61549;]]) to ablate the neurons of the patch compartment and allowed to recover for eight days. The animals were given daily injections of COC (25 mg/kg) or saline for one week, followed by a weeklong drug-free period. Animals were then given a challenge dose of COC, placed in activity chambers, observed for 2h and sacrificed. DERM-SAP pretreatment reduced the number of mu-labeled patches in the striatum. DERM-SAP pretreatment significantly reduced the intensity and spatial immobility of COC-induced stereotypy. In support of this observation, increased locomotor activity was seen in DERM-SAP pretreated, COC-treated animals. DERM-SAP pretreatment attenuated COC-induced c-Fos expression in the patch compartment, while enhancing COC-induced c-Fos expression in the matrix compartment. These data indicate that the patch compartment is necessary for repetitive behavior and suggests that alterations in activity in the patch vs matrix compartments may contribute to this phenomenon.

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506.24/M13. ***Contribution of stress to the effects of 5-HT1B receptor agonist on cocaine-induced locomotion before and after abstinence from repeated injections in C57BL/6 mice***

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We previously showed that 5-HT1B receptors (5-HT1BRs) modulate cocaine abuse-related behavior in opposite directions depending on the addiction cycle phase (i.e., maintenance vs. abstinence) in rats. Recently we showed that C57BL/6 mice treated daily with either saline (1 mL/kg, IP) or cocaine (15 mg/kg, IP) for 21 days exhibited different responses to test day pretreatment with the 5-HT1BR agonist CP94253 (CP) depending on whether the agonist was given on the last day of the chronic treatment or 2 days later. Specifically, CP increased locomotion on the last chronic treatment day but decreased locomotion when given 21 days later, and surprisingly this effect occurred regardless of whether the mice had received chronic cocaine or chronic saline. In this study we assessed whether the flip in the agonist effect on locomotion in chronic saline-treated mice was due to injection stress and/or housing with chronic cocaine-treated mice. Drug-naïve mice arrived at the same age as mice from our previous study. One group received NO INJECTIONS and were handled twice per week during which their tails were colored for identification. Another group was treated similarly except that they received daily repeated SALINE INJECTIONS at the same time of day for 21 days similar to our previous study. On the

last day of treatment, after a 1-h habituation period in test chambers, mice in both groups received either vehicle (1 mL/kg, IP) or CP (10 mg/kg, IP) and were returned to their home cage for 3 min. Next, mice were injected with saline or cocaine (5 mg/kg, IP) and placed immediately into test chambers for 1 h. The same test session was repeated after a 21-day period during which mice were handled twice per week to remark tails. Our results show that drug-naïve, No Injection mice, showed no change in locomotion in response to CP on either test day. However, chronic Saline Injection mice still exhibited a mild decrease in locomotion when pretreated with CP after a 21-day abstinence period. Literature suggests that chronic injections are a stressor in mice. We postulate that the decrease in locomotion in response to CP 21 days after ending chronic Saline Injections (present study) or chronic cocaine injections (previous study) likely involves the same mechanisms as reflected by cross sensitization between chronic injection stress and cocaine. The CP-induced attenuation effects of chronic saline stress and chronic cocaine suggests that 5-HT<sub>1B</sub>R agonists may have therapeutic potential for treating cocaine dependence.

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514.02/P15. ***AAV-mediated human arginine decarboxylase (hADC) overexpression modulates opioid tolerance and reinstatement of opioid self-administration***

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Background: Agmatine, decarboxylated L-arginine, has been shown to reduce opioid analgesic tolerance and opioid-induced drug addiction. Agmatine inhibits NMDA receptor dependent-behavior and spinal long term potentiation; the mechanism by which agmatine reduces pathological neuroadaptation likely involves that system. Since agmatine is endogenous, it may be possible to increase local agmatine levels by overexpression of its enzyme (arginine decarboxylase, ADC). We have developed an adeno-associated viral vector (AAV) to express human ADC in peripheral neurons and the CNS. We then compared development of intrathecal endomorphin-2 induced analgesic tolerance in subjects treated with vector (AAV-hADC) or saline. We also evaluated the impact of AAV9-hADC delivered to the nucleus accumbens in a model of opioid reinstatement. Methods: Endo-2 tolerance. Mice were pre-treated with either AAV5-hADC or saline. Endo-2 tolerance was induced acutely with 1 nmol intrathecal injection of Endo-2 or saline. Cumulative dose-response curves to Endo-2 were constructed in each of the groups (Saline-Saline, Saline-Endo-2, AAV5-hADC-saline, AAV5-hADC-Endo-2.) The impact of immunoneutralization of putative endogenously produced agmatine was assessed through the intrathecal pre-treatment of anti-agmatine IgG versus normal IgG. After testing, spinal cords were tested via HPLC analysis for agmatine content. Oxycodone Reinstatement. Mice were trained to lever press for oral oxycodone. Following abstinence, mice were injected with either AAV9-hADC or saline bilaterally in the nucleus accumbens. Reinstatement to oral oxycodone was assessed at 3 weeks post-injection. Results: rightward shift in the Endo-2 dose-response curve was observed in control subjects. However, in subjects pre-treated with AAV5-hADC, the ED<sub>50</sub> values were equivalent between the Endo-2 and

saline groups, showing lack of tolerance. In AAV5-hADC subjects pre-treated with anti-Ag IgG (but not normal IgG) Endo-2 tolerance developed, suggesting the importance of endogenous agmatine. Spinal levels of agmatine were elevated in the AAV5-hADC-treated subjects versus controls. Also, subjects treated with AAV9-hADC demonstrated a trend toward reduced reinstatement to oxycodone relative to those treated with saline. Conclusion: We interpret the observed inhibition of endomorphin-2 analgesic tolerance in the AAV5-hADC treatment, which was reversed by the pre-treatment of the anti-agmatine IgG, as attributable to agmatine produced from the overexpression of hADC. This is supported by the elevation in agmatine spinal cord levels in the hADC-treated subjects.

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514.04/P17. ***Study of neuropeptides involved in opioid induced hyperalgesia through liquid chromatography mass spectrometry***

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Opioid induced hyperalgesia (OIH) refers to a paradoxical nociceptive sensitization state caused by exposure to opioids which are used for the treatment of chronic pain. Although the precise molecular mechanism of OIH has not been fully characterized, changes in neuropeptides within the pain pathway are hypothesized to lead in OIH. The role of specific neuropeptides (eg. dynorphin) have been suggested, but there has not been a comprehensive peptidomics study of nervous system regions involved in OIH. To better understand the role of neuropeptides in OIH, we characterized the endogenous peptides in several critical brain regions involved in OIH using rodent model. OIH was established by injecting escalating dose of morphine twice daily for 4 days. Four different defined regions in the OIH biological circuits were isolated from rats, including the periaqueductal grey, nucleus accumbens, rostroventral medulla and dorsal horn of the spinal cord. Peptides were extracted from tissues and analyzed by nanoLC coupled to a high resolution Q-TOF mass spectrometer. PEAKS software was employed to perform database searches for peptide identification. Initially 43 neuropeptides derived from 11 prohormones have been identified. Opioid peptides play important roles in pain by interacting with opioid receptors. 12 opioid peptides were identified and pro-enkephalin derived peptides were detected in all the regions investigated. Other pain-related peptides were also detected, including substance P, short neuropeptide K and somatostatin (77-87) and pituitary adenylate cyclase-activating polypeptide (111-128). We also observed cerebellin in spinal cord which affects synapse plasticity, and may regulate the neural adaptations result in OIH. In addition, CGRP (19-37) was detected in the dorsal horn. This truncated CGRP has an arginine at the preceding position, which suggests it may not be a degradation product but a peptide enzymatically processed at the monobasic basic site. This work has laid the groundwork for exploring the role of neuropeptides in OIH. In the future, a comparative peptide quantitation study will be conducted between OIH and control groups to discover signaling peptides whose expressions are significantly influenced by OIH. Physiological experiments on the molecular level will help discover their functions and lead to a better understanding of the mechanism of this prevalent disease.

514.05/P18. ***Delta opioid receptor functional competence is inhibited by lipoxygenase metabolites in the carrageenan model of inflammatory pain***

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The function of delta opioid receptors (DOR) expressed by peripheral pain-sensing neurons (nociceptors) is regulated by both cyclooxygenase- (COX) and lipoxygenase (LOX)-dependent arachidonic acid (AA) metabolites. Unlike opioid receptors expressed in other areas (e.g. CNS) or in heterologous receptor systems, opioid receptors expressed by nociceptors are functionally inactive with respect to producing antinociception or inhibition of adenylyl cyclase activity. However, following brief exposure to inflammatory mediators such as bradykinin (BK) or AA, opioid receptors become responsive to opioid agonists (functionally competent) due to the actions of COX-dependent metabolite of AA. Moreover, in response to metabolism of AA by LOX, DOR returns to an unresponsive state that is refractory to re-induction of functional competence (Sullivan et al., 2015, J Pharmacol Exp Ther 353(1):44-51). Here we show that DOR functional competence can also be induced in vivo by brief exposure to carrageenan. Carrageenan produces a state of inflammation that involves a variety of inflammatory mediators, including BK and AA. Intraplantar (i.pl.) injection of carrageenan (500 µg) produced thermal allodynia that lasted for at least 24 h. When tested 15 min after carrageenan, injection of the DOR agonist, DPDPE (20 µg, i.pl.) blocked carrageenan-induced thermal allodynia, indicating that DOR was functionally competent. When tested 3 h or 24 h after carrageenan, DPDPE did not inhibit thermal allodynia indicating that DOR had become unresponsive. However, following injection (i.pl.) of the 12- and 15-LOX inhibitors, Luteolin (3 µg) and Baicalein (3 µg), responsiveness to DPDPE (DOR functional competence) at the 3 h and 24 h time points was restored. These data indicate that opioid receptor system functional competence induced in response to local inflammation produced by carrageenan is transient. However, similar to our findings with AA-mediated induction of DOR functional competence, the duration of functional competence can be increased by inhibition of LOX, suggesting that a LOX-dependent AA metabolite produces an unresponsive state of DOR. Results of this study further underscore the extraordinary regulation of the function of opioid receptors expressed by peripheral sensory neurons. Peripherally-restricted opioids may be very effective at treating pain due to inflammation.

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514.06/P19. ***6'-Guanidinonaltrindole (6'-GNTI) targets DOR-KOR heteromers in peripheral sensory neurons***

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Originally developed as a KOR agonist, 6'-GNTI has been shown to have agonist activity at KOR for Gi-protein-mediated signaling in brain and HEK cells and affinity (but no efficacy) for DOR. However, in peripheral nociceptors, we have found that 6'-GNTI agonist activity for Gi-mediated responses requires

expression of both KOR and DOR. In primary cultures of peripheral sensory neurons, 6'-GNTI inhibited adenylyl cyclase activity by 65%. However, following siRNA knockdown of DOR, 6'-GNTI had no effect on adenylyl cyclase activity but antagonized the response to the KOR agonist, U50488. Similarly, when KOR expression was reduced with siRNA treatment, 6'-GNTI had no effect on adenylyl cyclase activity, but antagonized the response to the DOR agonist, DPDPE. Thus, 6'-GNTI has affinity, but not efficacy (i.e. acts as an antagonist) when either DOR or KOR expression is reduced. Importantly, these data suggest that 6'-GNTI efficacy requires activation of DOR-KOR heteromers in peripheral nociceptors. Because we have demonstrated that allosteric interactions between protomers of DOR-KOR heteromers can regulate DOR and KOR agonist potency and efficacy in peripheral nociceptors, (Berg et al., 2012, Mol Pharmacol 81:264-272; and Jacobs et al this meeting), we next tested the hypothesis that 6'-GNTI occupancy of the DOR protomer of DOR-KOR heteromers allosterically enhances its own efficacy at KOR. In peripheral sensory neuron cultures, we measured 6'-GNTI-mediated responses in the presence of several different selective DOR antagonists. Both inhibition of adenylyl cyclase activity as well as antinociception in response to 6'-GNTI were reduced in the presence of the DOR antagonists naltrindole (2nM, 100x Ki) or 7-Benzlidenealtrexone (1nM, 100x Ki). By contrast, naltriben (NTB, 1nM, 100x Ki), fully substituted for 6'-GNTI occupancy of DOR. The concentration response curves of 6'-GNTI for inhibition of adenylyl cyclase activity were superimposable in the absence (DOR occupancy by 6'-GNTI) and presence of NTB (DOR occupancy by NTB). Similarly, in a behavioral model of thermal allodynia, 6'-GNTI produced the same robust antinociceptive response in the presence or absence of NTB. These data are consistent with the hypothesis that 6'-GNTI occupancy of DOR augments its own efficacy at KOR through allosteric interactions between DOR and KOR within the DOR-KOR heteromer.

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514.07/P20. ***Prolonged functional competence of delta opioid-kappa opioid receptor (DOR-KOR) heteromers in the rat carrageenan model of inflammatory pain***

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Opioid receptor systems expressed by peripheral pain-sensing neurons (nociceptors) are under dual regulatory control by cyclooxygenase (COX) and lipoxygenase (LOX) dependent arachidonic acid (AA) metabolites. For example, delta opioid receptors (DOR) are functionally inactive under basal conditions, but become responsive (i.e., functionally competent) following exposure to inflammatory mediators (e.g., carrageenan, bradykinin (BK) or AA) that produce COX-dependent AA metabolites. Following induction of functional competence by AA, DOR reverts to a basal non-responsive state that is refractory to re-induction of functional competence. This refractory, non-responsive state can be blocked by inhibiting LOX thereby allowing DOR functional competence to be re-induced (Sullivan et al., 2015, J Pharmacol Exp Ther 353: 44-51). Recently we reported that, in peripheral nociceptors, DOR forms heteromers with kappa opioid receptors (KOR) that produce robust antinociceptive responses following exposure to BK (Berg et al., 2012, Mol Pharmacol 54:94-104). Here we sought to explore DOR-KOR

heteromer responsiveness in the carrageenan model of inflammatory pain. We examined the actions of the DOR agonist, DPDPE, the KOR agonist, U50488 and the DOR-KOR heteromer agonist, 6'-GNTI, to inhibit carrageenan induced thermal allodynia in the rat. When tested 15 min after intraplantar (i.pl) injection of carrageenan (500 ug), all agonists were effective at reducing carrageenan-induced thermal allodynia. When tested either at 3h or 24h post-injection of carrageenan, neither DPDPE nor U50488 reduced the thermal allodynia. However, responsiveness (i.e. functional competence) was restored by inhibition of LOX. Interestingly and by contrast, 6'-GNTI remained capable of inhibiting carrageenan-induced thermal allodynia for up to 24h (longest period tested) post-injection. These data suggest that DOR-KOR heteromers are differentially regulated by LOX metabolites. Further, in striking contrast to DOR and KOR, DOR-KOR heteromers appear to remain functionally competent for prolonged period of time under inflammatory conditions, suggesting that they may be suitable targets for development of peripherally-restricted pain medications.

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514.08/P21. ***Epigenetic regulation of spinal cord gene expression contributes to enhanced postoperative pain and analgesic tolerance after continuous opioid exposure***

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Opioid induced hyperalgesia (OIH), as well as, analgesic tolerance are unfavorable consequences of extended opioid use. Both typically resolve within days after cessation of morphine treatment. Post-surgical pain is prolonged if mice are previously exposed to opioids. We have shown earlier that among several implicated gene targets, expression levels of Bdnf (Brain-derived neurotrophic factor) and Pdyn (Prodynorphin) were closely related to OIH. The present study was carried out to investigate the epigenetic regulatory changes of Bdnf and Pdyn in supporting the enhanced incisional pain hypersensitivity after opioid exposure. The study went on to characterize morphine analgesic tolerance at several timepoints following surgery in groups exposed to morphine earlier. Mice were treated with ascending doses of morphine for 7 days and subsequently received hind paw incisions. Mechanical withdrawal thresholds were significantly decreased in the morphine plus incision group compared to morphine or incision alone groups. Analgesic tolerance to morphine was increased on days 3 and 6 after surgery in the morphine plus incision group compared to respective controls. Expression of Bdnf and Pdyn were increased on day 3 but only Pdyn levels were elevated on day 6 after surgery. CHIP (Chromatin immunoprecipitation) assays demonstrated that promoter regions of Pdyn and Bdnf were more strongly associated with acH3K9 (Acetylated histone H3 Lysine9) after morphine plus incision treatment than morphine or incision alone groups. The selective TrkB (tropomyosin-receptor-kinase) antagonist ANA-12 reduced hyperalgesia when given spinally one or three days after surgery. Intrathecal treatment with the selective kappa opioid receptor antagonist nor-BNI had similar effects on pain sensitivity. The administration of ANA-12 or nor-BNI attenuated morphine analgesic tolerance on day 1, but only nor-BNI was effective on day 3 after surgery in opioid exposed group. The co-administration of histone acetyltransferase inhibitor anacardic acid daily with morphine resulted in reversal of OIH and

further treatment attenuated enhanced hyperalgesia in the morphine plus incision group compared to controls. The present study shows histone modification of spinal genes contributes to enhanced postoperative pain and analgesic tolerance after continuous opioid exposure. Treatments blocking the differential expression of BDNF and dynorphin by inhibiting histone acetylation would have a potential in reducing postoperative pain, OIH and tolerance.

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514.09/P22. ***An allosteric modulator of the mu-opioid receptor promotes opioid-mediated antinociception***

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Positive allosteric modulators (PAMs) of the mu-opioid receptor, such as BMS-986122, are compounds that bind to a site on the receptor that is distinct from the orthosteric site for endogenous opioid peptides and traditional opioid analgesics and enhance the activity of orthosteric agonists. Such mu-opioid receptor PAMs (mu-PAMs) could act as stand-alone analgesics and/or enhance the action of traditional opioid agonists and thereby reduce the level of opioid drug required to afford pain relief. In cells expressing the mu-receptor BMS-986122 is silent, i.e. it does not activate signaling downstream of the mu-opioid receptor. However, in membranes from both cloned cells expressing mu-opioid receptors and mouse brain membranes BMS-986122 enhances the binding affinity and potency and/or efficacy of wide range of mu-opioid receptor orthosteric agonists, including endogenous opioid peptides, in a probe-dependent manner. The most robust increase in affinity and potency is seen with R(-)-methadone. Here we provide proof of principle that mu-PAMs can be effective in vivo by determining antinociceptive activity in the mouse using the hot-plate assay. In addition, opioid modulation of GABA synaptic transmission was monitored in periaqueductal gray (PAG) neurons. BMS-986122 given intracerebroventricularly (i.c.v.) caused a substantial increase in the antinociceptive activity of R(-)-methadone given i.c.v. or systemically and a moderate increase in the antinociceptive activity of systemic morphine. At higher doses BMS-986122 alone (i.c.v.) afforded short-lived antinociception that was completely prevented by pretreatment of the mice with naloxone or the selective mu-antagonist beta-FNA. Furthermore, BMS-986122 promoted swim-stress induced antinociception that was blocked by pretreatment with naloxone. In slices of the PAG, BMS-986122 enhanced the ability of Met-enkephalin to inhibit presynaptic GABA release. These studies demonstrate that mu-PAMs enhance the activity of the mu-opioid receptor in vivo and so increase the antinociceptive activity of opioid drugs and endogenous opioid peptides acting at the mu-opioid receptor.

514.16/P29. ***The delta opioid receptor agonist SNC80 preferentially recruits beta-arrestin to promote analgesic tolerance***

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Ligand directed signaling via the delta opioid receptor (DOR) has important implications given the potential therapeutic uses of delta agonists in the treatment of chronic pain and emotional disorders. We had previously shown that repeated injection of the high-internalizing delta agonist (+)-4-[( $\alpha$ R)- $\alpha$ -((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide (SNC80), produced acute behavioral desensitization while the low-internalizing delta agonist N,N-diethyl-4-(phenyl-piperidin-4-ylidenemethyl)-benzamide (ARM390) did not. Since beta-arrestins are well known to regulate G protein-coupled receptors signaling and trafficking, we therefore investigated the behavioral significance of ligand-specific interactions between beta-arrestin and the DOR. Mice lacking beta-arrestin showed enhanced and longer lasting pain-relieving effects of SNC80, and decreased acute tolerance following repeat exposure to the agonist. In contrast, ARM390 produced similar analgesic effects and no acute tolerance in both wildtype and knockout animals. Following chronic treatment, the absence of beta-arrestin attenuated the extent of tolerance to SNC80, but not to ARM390. Furthermore, chronic treatment with SNC80 abolished delta agonist-induced GTP $\gamma$ S binding in wildtype brain membranes, whereas DOR-G protein coupling remained intact in beta-arrestin 1 knockout mice. Overall, these results indicate that delta opioid receptor agonists interact with beta-arrestins in ligand-biased manner, and that the high-internalizing agonist SNC80 preferentially recruits beta-arrestin 1.

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514.20/P33. ***Targeting putative mu opioid/chemokine receptor type heteromers potently attenuates nociception in a murine model of chemotherapy-induced peripheral neuropathy***

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Although powerful, pharmacological antineoplastic therapies has led to increased survival for millions of cancer patients, chemotherapeutic agents such as cisplatin produce a number of serious side effects, including peripheral neuropathy. Chemotherapy-induced peripheral neuropathy (CIPN) causes sensory disturbances in the extremities including numbness, paresthesia, and pain, and is the primary dose-limiting side effect that reduces efficacy of treatment and ultimately affects survival. Analgesics typically used to treat neuropathic pain appear to be relatively ineffective in alleviating pain from CIPN as well as display their own display dose-limiting adverse effects, such as those associated with opiates. Given the requisite need for superior pharmacological agents in its treatment, we investigated the

antihyperalgesic effects of a novel bivalent ligand, MCC22, in a murine model of cisplatin chemotherapy-induced neuropathic pain. MCC22 contains both mu agonist and CCR5 antagonist pharmacophores linked through a 22 atom spacer. Given the existence of opioid receptor heteromers in cultured cells and possibly in vivo, MCC22 may be a potent alternative for the treatment of neuropathic pain without attendant side effects. Adult male C3H/HeJ mice were tested for mechanical paw withdrawal responses on 2 consecutive days prior to treatment by determining the frequency of withdrawal evoked by a calibrated von Frey monofilament with a bending force of 3.9 mN applied to the plantar surface of the hind paws. Mice were then given 1 mg/kg of cisplatin intraperitoneal (i.p.) daily for 7 consecutive days and on day 1 post-injection, mechanical hyperalgesia was assessed. Compounds were administered intrathecally (i.t.) and i.p. weekly to determine peak time effects and ED50/80. Compared to the antihyperalgesic effectiveness of the standard opioid agonist morphine, we evaluated the effects of MCC22 both i.t. and i.p. and found MCC22 given i.t. (ED50: 0.0004 pmol/mouse (0.0002-0.0009 95% CI) to be significantly more potent than i.t. morphine (ED50: 27.44 pmol/mouse (16.57-45.43 95% CI) with a peak time of 20 minutes. MCC22 exhibited no tolerance and increased in potency over 115 days. It was also found to be significantly more potent when given i.p.(ED50: 3.07 pmol/mouse (2.45-3.84 95% CI) compared to morphine (ED50: 14.28 pmol/mouse (12.70-16.07 95% CI) with peak time of 3 minutes and again did not exhibit tolerance. Morphine both i.t. and i.p. showed significant tolerance. MCC22 potently attenuates hyperalgesia in a cisplatin model of neuropathy and may offer a viable treatment for patients who suffer from CIPN pain without concomitant side effects.

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538.02/BB76. ***The role of median raphe GABA and glutamate neurons in reward***

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Previous pharmacological research has shown that rats will self-administer GABA receptor agonists, AMPA receptor antagonists, and NMDA receptor antagonists into the median raphe region (MR). However, there are diverse populations of neurons\_serotonergic, glutamatergic, and GABAergic\_in the MR, and the roles they play in reward are unclear. To resolve this issue, the present study utilized optogenetics to selectively excite or inhibit particular populations of MR neurons. Wild-type C57 mice received a viral vector encoding halorhodopsin (NpHR) in the MR, and learned to self-administer photostimulation, suggesting that net inhibition of MR neurons is rewarding. Transgenic vGat-Cre mice received NpHR in the MR, resulting in selective expression of the opsin in GABA neurons. These mice learned to self-administer photostimulation at a greater level than C57 mice, suggesting that inhibition of GABA neurons is one part of the circuitry underlying MR reward. Transgenic vGluT3-Cre mice received channelrhodopsin (ChR2) in the MR, resulting in selective expression of the opsin in glutamate neurons. These mice also learned to self-administer photostimulation at a greater level than C57 mice, suggesting that excitation of glutamate neurons is another part of the circuitry underlying MR reward. Taken together, inhibition of MR GABA neurons may disinhibit MR glutamate projection neurons, which

activate global reward circuits. The MR may be another important region to study in order to understand the neurobiology of affect and addiction.

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633.04/CC8. ***Measuring and manipulating corticostriatal functional neural circuitry in the socially monogamous prairie vole***

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The ability to form positive social relationships is key to mental health, and yet the underlying functional neural circuitry remains poorly understood. The socially monogamous prairie vole is a canonical animal model for social bonding. Previous anatomical, genetic, and pharmacological studies have implicated two corticostriatal nodes [[unable to display character: &#8211;]] the medial prefrontal cortex (mPFC) and nucleus accumbens (NAcc) [[unable to display character: &#8211;]] in vole social bond formation. However, these approaches do not provide a dynamic view of the functional neural activity and connectivity of these regions during social interactions leading to a bond. To address this, we measured and manipulated neural activity within this circuit in socially-behaving female voles. We found an enhancement in low-frequency mPFC-to-NAcc connectivity during mating, a behavior that accelerates vole bond formation, compared to the control, non-social behavior of self-grooming. Further, optogenetically stimulating mPFC afferents to the NAcc at low frequencies in the absence of mating shifts later behavioral preference towards a partner, suggesting that low-frequency activation of this circuit is functionally relevant for bond formation. Finally, phase-amplitude coupling from mPFC to NAcc is enhanced during mating, suggesting that mPFC activation during social bonding drives NAcc by rhythmically modulating its excitability. Together, these results reveal a dynamic picture of corticostriatal activation during bond formation, with exciting implications for how affiliative social interactions can recruit reward and reinforcement systems to drive changes in behavior. A key ongoing direction is to determine the role of neurochemicals (e.g. oxytocin) in modulating this system.

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695.08/N19. ***Alcohol addiction impairs human hippocampal neurogenesis: Effects o proliferation, neuronal stem cells and immature neurons***

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Alcohol abuse is associated with neurodegeneration in the hippocampus, a region associated with learning, memory and mood regulation in humans. We hypothesized that alcohol may have detrimental

effect on the neurogenic pool of stem cells and/or immature neurons in the dentate gyrus (DG) of the human hippocampus. Therefore, we investigated whether alcohol abuse affects the number of neuronal stem/progenitor cells, immature/migrating neurons and proliferating cells in postmortem human hippocampal samples isolated from deceased donors subjected to forensic autopsy. Classification of subjects was based on amount of alcohol consumed the last 4 weeks before their demise, according to information from relatives, the forensic pathology investigations, police reports and medical records. Hippocampal sections from controls and alcoholics were immunostained for Sox2, neuronal stem/progenitor cell marker, doublecortin, a marker for immature/migrating neurons, and Ki67, a marker of cell proliferation. Positively stained cells were counted in alcoholics and compared with age-matched controls. Counting was performed in whole DG, including the molecular layer (ML), the granular cell layer (GCL) and the subgranular zone (SGZ). We also counted cells separately in the SGZ. The number of cells immunoreactive for doublecortin, Sox2 and Ki67 was significantly reduced in the whole DG and in the SGZ in alcoholics as compared to controls. No correlation between the cell numbers expressing any of the three markers and subject age was observed. In summary, our data indicate that alcohol impairs neurogenesis in the human hippocampus, and suggest that pharmacological agents that act on the hippocampal stem cell pool may be particularly interesting to study in the search for drugs to treat alcohol addiction.

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695.10/N21. ***Chronic ethanol self-administration in female macaques disrupts presynaptic dopamine neurotransmission***

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Hypofunction of striatal dopamine neurotransmission, or hypodopaminergia, is a consequence of excessive ethanol use, and is hypothesized to be a critical component of alcoholism, driving alcohol intake in an attempt to restore dopamine levels; however, the neurochemical mechanisms involved in producing dopamine signaling deficiencies are unknown. Here we examined the specific dopaminergic adaptations induced by chronic ethanol self-administration that produce hypodopaminergia and may contribute to alcohol use disorders. Female rhesus macaques (3 controls, 5 drinkers) completed one year of daily (22 hr/day) voluntary ethanol self-administration. Animals were given ethanol access until 3.5-6.5 hours before ex vivo fast-scan cyclic voltammetry in post-mortem brain slices containing the nucleus accumbens core was used to determine ethanol-induced alterations in dopamine terminal function including dopamine release and uptake kinetics as well as the ability of quinpirole (D2/D3 dopamine receptor agonist) and U50,488 (kappa-opioid receptor agonist) to inhibit dopamine release. Chronic ethanol drinking increased dopamine uptake rates, and uptake rates were positively correlated with lifetime ethanol intake. Further, the sensitivity of inhibitory D2/D3 dopamine autoreceptors and

kappa-opioid receptors were also enhanced following drinking. Together, these factors likely converge to drive a hypodopaminergic state, characterized by an inability to mount an appropriate dopaminergic response to salient stimuli. Additionally, these data suggest that the dynorphin/kappa-opioid receptor system may be an efficacious target for pharmacotherapeutic interventions in the treatment of alcohol use disorders.

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695.12/N23. ***Neurosteroids modulate ethanol effects on dopamine release in the nucleus accumbens via actions on GABA(A) receptors on VTA GABA neurons***

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Dopamine (DA) transmission in the mesolimbic reward system originating in the ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAc) is lowered during withdrawal from chronic ethanol exposure. Levels of neurosteroids with GABA(A) receptor-modulating properties are also lowered in the VTA by chronic ethanol, suggesting that select neurosteroids may be targets for restoring VTA DAergic function. The primary regulation of VTA DA neuron excitability is mediated by GABA(A)-mediated inhibition, presumably from local circuit VTA GABA neurons. We have shown in multiple reports that VTA GABA neurons are inhibited by acute ethanol and become hyperexcitable during withdrawal from chronic ethanol, presumably due to a switch in GABA(A) receptor function on VTA GABA neurons. VTA GABA neurons inhibit DA release either through local circuit inhibition or via projections to DA terminals in the NAc. The goal of this study was to determine the involvement of GABA(A) modulating neurosteroids in VTA GABA neuron excitability and in DA neurotransmission in the mesolimbic pathway. Using fast scan cyclic voltammetry (FSCV), we performed experiments on brain slices in the NAc by superfusing various GABA(A)R-modulating neurosteroids - allopregnanolone, DHEAS, and estrone sulfate. We also tested Trilostane, which enhances the endogenous expression of DHEAS via block of 3 $\beta$ -hydroxysteroid dehydrogenase. DHEAS (20  $\mu$ M) enhanced DA release in the NAc by 2 %, while allopregnanolone, estrone sulfate, and Trilostane did not significantly alter DA release. Ethanol (20 - 160 mM, IC50 = 80 mM) reduced DA release in wild type (WT) mice. Superfusion of DHEAS (20  $\mu$ M) significantly attenuated ethanol inhibition of DA release. However, the other neurosteroids did not significantly alter ethanol inhibition of DA release. In electrophysiological single-unit studies in vivo, DHEAS markedly enhanced VTA GABA neuron firing rate. Experiments are in progress to further evaluate the effects of the GABA(A) receptor-modulating neurosteroids on VTA and NAc neuron firing rate as well as GABA(A) receptor-mediated synaptic transmission to VTA GABA neurons.

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695.17/N28. ***Alcohol-induced changes in cannabinoid modulation of noradrenergic neurons***

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Alcohol use disorders (AUDs) affect approximately 8.5% of American adults, and while remission is achievable, longitudinal data indicate that 60-90% of alcoholics will relapse within their first four years of abstinence. Moreover, sex differences related to alcohol's negative effects are emerging. For example, chronic alcohol exposure causes increased negative physiological effects on bodily organs and increases feed-forward activation of the hypothalamo-pituitary-adrenal axis in females when compared to males. One major symptom of alcohol withdrawal is increased stress-induced anxiety, which can often lead to relapse in recovering alcoholics. The locus coeruleus (LC) provides the sole source of norepinephrine (NE) to the frontal cortex and high levels of cortical NE have been implicated in the pathophysiology of stress-related psychiatric disorders. Via corticotropin-releasing factor (CRF) neurotransmission, stress exposure activates LC neurons, increasing the release of NE in forebrain targets. Chronic alcohol exposure alters CRF activation of LC-NE neurons and subsequently impacts NE release. The endocannabinoid (eCB) system regulates neurotransmitter release, and emerging studies suggest targeting the eCB system may influence the development of anxiety and stress-induced psychiatric disorders. Taken together, actions of CRF and eCBs may converge in the LC to modulate stress-induced anxiety responses. However, the effect of alcohol on this interaction remains unknown. Here, we investigated the effect of repeated alcohol administration on cannabinoid type 1 receptor (CB1r) in the LC using Western blot analysis. Male and female Sprague-Dawley rats were match-pair fed calorically equivalent liquid diet containing 36% of calories as ethanol for 1 days to induce an ethanol-addicted state. Alcohol treated females demonstrated a significant decrease in CB1r levels when compared to control and male counterparts ( $p < 0.05$ ). We also expanded on prior results showing co-existence of CB1r and CRF by examining whether CB1r/CRF afferents were excitatory or inhibitory in nature. Triple immunofluorescence microscopy showed that CB1r/CRF afferents expressed either the vesicular glutamate transporter or glutamic acid decarboxylase in the LC. These studies suggest that alcohol causes greater impact on the eCB system in females compared to males in the stress-integrative LC and that CB1r can regulate CRF release in afferents that express excitatory and inhibitory amino acids.

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695.29/N40. ***History of drug intake leads to compulsive appetite via disruption in non-homeostatic control of food intake***

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While the 'food addiction' model of pathological overeating has been popularized, fundamental shortcomings of this nosology still remain to be addressed. Nevertheless, striking similarities in

behavioral manifestations and their neurobiological underpinnings exist between substance dependence and eating disorders. We hypothesized that a history of extensive drug or alcohol intake known to result in 1) addiction-like drug motivation and 2) addiction-linked brain changes would result in similar addiction-like motivation for food in rats. Initially, different groups of rats were trained to self-administer cocaine or saccharin (controls). All rats were then given opportunities to self-administer sweetened condensed milk (SCM). The history of cocaine intake led to addiction-like SCM seeking and taking, marked by heightened resistance to 1) increased workload, 2) extinction, and 3) adverse consequences. However, a history of extensive cocaine intake failed to alter subsequent 1) bodyweight gain, 2) ad libitum chow intake 3) and, interestingly, ad libitum consumption of a highly palatable (high fat/ high sugar) diet. Because the addiction-triggering history of extensive drug intake is insufficient to induce overeating per se, the addiction-like motivation for SCM or 'compulsive appetite' observed in these rats is likely due to dysregulation of non-homeostatic (non-metabolic) rather than homeostatic (metabolic) control of food intake. Consistent with this hypothesis, two additional groups of rats with either cocaine or alcohol history exhibited heightened resistance to an electric footshock paired with the delivery of a non-caloric saccharin reward. Moreover, a history of cocaine intake induced functional upregulation of group II metabotropic receptors (mGluR2/3) in medial prefrontal cortex (mPFC) and amygdala; brain sites implicated in non-homeostatic control of food intake. These receptors negatively modulate neural excitability via inhibitory Gi proteins and, thus, may contribute to impaired functional connectivity between mPFC and amygdala observed in drug addicts. Together, the nosology of addiction is most applicable to certain phenotypes of eating disorders characterized by compulsive appetite, such as binge-eating disorder and bulimia nervosa, rather than pathological overeating in general. Neurobiological irregularities in the non-homeostatic pathway - abnormalities akin to those observed in drug addicts - are likely to provide the neuroregulatory basis for compulsive appetite.

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**730.03/CC7. Local network differences in reactive aggression measured with resting-state fMRI and graph theory**

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Reactive aggression is characterized by excessive anger expression and limited anger control. Even when unchallenged by a task or provocation, individuals with trait patterns of these anger characteristics are predicted to have differences in resting-state functional connectivity as compared to non-angry controls. Here we used graph theory, a mathematical approach for studying complex network properties, in a matched clinical sample (age, education, and race) of 12 individuals with reactive aggression (RA) in comparison to 12 low aggressive controls (LA) who completed resting-state functional magnetic resonance imaging and the State/Trait-Anger Expression Inventory (STAXI-II). As expected, groups differed in the outward expression of anger and anger control ( $p < 0.001$ ) subscales. We found decreased

clustering coefficient (the number of connections directly connected to a node over the number of possible connections for that node), a measure of the probability of connectedness between neighboring nodes, in the Sensorimotor Network (pre-motor cortex, mid-cingulate cortex, paracentral lobule, and dorsal precuneus), and visual superior occipital cortex in RA relative to LA ( $p < 0.05$ , FDR-corrected). Correlations across groups between the clustering coefficient connectivity properties of the Sensorimotor Network with outward expression of anger and anger control revealed that decreased local connectedness of the mid-cingulate cortex and paracentral lobule was linked to increased outward expression of anger ( $p$ -corrected=0.001), and decreased local connectedness of the paracentral lobule and dorsal precuneus was linked to lower anger control ( $p$ -corrected $<0.005$ ), ( $p$ -threshold=0.006; 4 regions  $\times$  2 trait variables). Disrupted local connectedness of the Sensorimotor Network with neighboring regions in R hints at deficits in monitoring, planning and control of motoric responses, and may predispose individuals prone to reactive aggression to act upon less-integrated and less-regulated reactions to salient environmental cues. This is further suggested by the relationship between decreased local connectivity in this network and increased outward expression of anger and lower anger control. We also provide a graph theoretical framework for integrating findings from previous studies showing an association between higher precuneus activity and higher negative emotionality and lower self-control in individuals with high trait aggression. Overall, lower local connectivity of sensorimotor regions may be a contributing factor for greater expression of anger and lower anger control in reactive aggression.

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730.06/CC10. ***Why Adaptive Coding? Signal and noise in neural transmission and adaptive coding in economic choices***

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In the context of economic decisions, adaptive coding refers to the fact that neurons encoding the subjective value of a given option adjust their firing rate to the range of values available in the environment. Adaptive coding has been found in the orbitofrontal cortex (Padoa-Schioppa, 2009, Kobayashi et al 2010), amygdala (Bermudez and Schultz, 2010), anterior cingulate cortex (Cai and Padoa-Schioppa 2012), ventromedial prefrontal cortex and ventral striatum (Cox and Kable, 2013). Adaptive coding has also been observed in dopamine cells encoding reward prediction errors (Tobler et al, 2005). All these studies found that the neuronal activity slope is larger when the range of values is smaller, while the range of firing rates remains constant across different environments. Importantly, adaptive coding can introduce biases in decision making (Padoa-Schioppa and Rustichini, 2014). Thus a fundamental question is: What (if anything) does the choice system gain from adaptive coding? Here we show that the slope adjustment induces an improvement in the speed-accuracy trade-off, and we provide a quantitative estimate of this gain. The improvement is essentially because the process governing signal transmission across neuronal layers has a drift proportional to the firing rate of pre-

synaptic cells, but standard deviation proportional to the square root of that firing rate. Everything else equal, and assuming linear encoding of values, increasing the slope affords faster and/or more accurate decision. The speed-accuracy trade-off is regulated by the stopping policy, which may depend on the environment and the details of the rules allocating rewards. We apply this general result to the several neural models of economic decisions, including the Drift Diffusion model (Ratcliff, 1978), the Leaky Competitive Accumulation model (Usher and McClelland, 2001), and several versions of the Pooled Inhibition model (Wang 2002; Wong and Wang, 2006). The general result that adaptive coding affords better set of speed and accuracy is robust to the specific model, and we estimate this gain. We also show that in non-linear decision models the accuracy may be a non-monotonic function of the input slope. Thus the limited activity ranges typically recorded in orbitofrontal cortex and other brain regions may be set to optimize decisions, and not be due to intrinsic physiological bounds.

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730.07/CC11. *neuro-computational model of economic decisions*

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Lesion studies and neurophysiology indicate that key aspects of economic decisions take place in the orbitofrontal cortex (OFC). Specifically, single-cell recordings in monkeys choosing between different juices identified in this area three groups of neurons: offer value cells encoding the value of individual goods, chosen juice cells encoding the binary choice outcome, and chosen value cells encoding the value of the chosen good. An important and open question is whether and how decisions could emerge from a neural circuit formed by these three populations. Here we adapted a biophysically realistic neural network previously proposed for perceptual decisions (Wang 2002; Wong and Wang 2006). The domain of economic decisions is significantly broader than that for which the model was originally designed, because offers vary independently of each other whereas coherence in the random-dot task is a 1D parameter: Yet the model performed remarkably well. The input and output nodes of the network (OV and CJ cells) were naturally mapped onto two groups of neurons in OFC (offer value and chosen juice cells, respectively). Surprisingly, the activity of interneurons in the network (CV cells) closely resembled that of the third group of neurons, namely chosen value cells. This resemblance reflect the fact that inhibitory interneurons in Wang's model receive the input from the two types of CJ cells. The model reproduced several phenomena related to the neuronal origins of choice variability including choice hysteresis, the "predictive activity" of chosen juice cells and the "overshooting" of chosen value cells (see Padoa-Schioppa, 2013). It also generated testable predictions on the excitatory/inhibitory nature of different neuronal populations and on their connectivity. Some aspects of the empirical data were not reproduced, but simple extensions of the model could overcome these limitations. These results render a biologically credible model for the neuronal mechanisms of economic decisions. They demonstrate that choices could emerge from the activity of cells previously identified in the OFC, suggesting that chosen value cells directly participate in the decision process. Importantly, Wang's model provides a platform to investigate the implications of neuroscience results for economic theory.

730.08/CC12. ***Neural activity in basolateral amygdala encodes reward magnitude and risk of punishment in risky decision-making task in rats***

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Elevated levels of risk-taking behavior are characteristic of substance addiction, and have the potential to precipitate and exacerbate substance use. A potential treatment strategy for addiction could be to attenuate such maladaptive choice behavior, so as to mitigate drug-seeking and potential relapse. To realize this goal, however, a thorough understanding of the neurobiology underlying normal risk-taking behavior is required. Using a rodent model of risk-taking, in which rats choose between a small, “safe” reward and a large, “risky” reward accompanied by a variable probability of punishment, we showed previously that an intact basolateral amygdala (BLA) is critical for integration of risk- and reward-related information to guide adaptive risk-taking. In the present experiment, we evaluated how neural activity in the intact BLA encodes risk- and reward-related information during task performance. Rats were trained in a modified version of the risky decision-making task in which reward magnitude and probability of punishment (50%) were independently manipulated in separate blocks of trials. During stable performance, rats chose the large reward significantly more than the small reward and chose the reward associated with risk of punishment significantly less than the reward associated with no punishment. Two drivable microwire bundles were then implanted immediately above the BLA. Upon recovery, neural activity was recorded while rats were tested in the risky decision-making task. Initial analyses indicated that during reward magnitude trials, there was an increase in BLA activity in anticipation of the large reward and decrease in activity in anticipation of the small reward relative to baseline. A difference was also observed during risk of punishment trials, such that BLA activity increased in anticipation of the risky reward and decreased in anticipation of the safe reward relative to baseline. Importantly, the reward magnitude associated with each choice during the risk of punishment trials was held constant, indicating that differences in firing were due to encoding of risk-related information. Together, these data suggest that BLA neurons track the most salient option during choice behavior. Future work will compare BLA neural activity in this task between drug-naïve and cocaine-exposed rats to determine whether dysfunctional BLA activity contributes to drug-induced maladaptive risk-taking behavior.

730.25/CC29. ***Neural evidence of good-based economic choice under varying action costs***

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Previous work showed that economic decisions can be made independently of the spatial configuration of the offers and independently of the action necessary to implement the choice (in goods space). However, goods available for choice may be associated with different action costs. In such conditions, the decision process must necessarily take into account some aspect of the action. Two schemes have been proposed to account for decisions under variable action costs. One possibility is that the brain first computes the "stimulus value" of each option and then combines the stimulus value with the corresponding action cost in an action-based representation. In this scheme, decisions are action-based and take place in premotor regions (Rangel and Hare, 2010). Alternatively, action costs could be integrated with other determinants of value (commodity, quantity, etc.) in an abstract representation. In this view, decisions under variable action costs could take place in the space of goods (Padoa-Schioppa, 2011). To shed light on this fundamental issue, we recorded from the orbitofrontal cortex while monkeys chose between different juices offered in variable amounts and with variable action costs. Specifically, we manipulated the cost associated to each offer by varying the amplitude of the saccade necessary to indicate the chosen option. At the beginning of each trial, the animal fixated the center of a computer monitor. Two offers then appeared on the two sides of the fixation point. Each offer was represented by a set of color symbols. Different colors represented different juices, the number of symbols represented the juice quantity, and the shape of the symbols represented the saccade amplitude (short or long). Offers remained on the monitor for  $s$ , followed by  $s$  delay. Two saccade targets then appeared on the monitor. The color of each saccade target was that of the corresponding juice, the radial distance from the center fixation was set accordingly to the action cost, and the angular position of the two targets was chosen randomly on each trial. After a go signal, the animal indicated its choice with a saccade. Critically, this design provided the opportunity to dissociate in time the decision from the formation of an action plan. We recorded the activity of 754 cells. Different groups of neurons encoded the offer value, the chosen value and the identity of the chosen option. Remarkably, both juice-based and cost-based neuronal representations were present at the same time. Furthermore, chosen juice and chosen cost neurons encoded the choice outcome well before the presentation of saccade targets, indicating that economic decisions were made in goods space.

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777.19/13. ***Onset-specific effects of regular cannabis use on brain functional systems***

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Graph theory models propose that brain regions have a pattern of functional connections that form a brain functional system. Thus, graph theory provides a more holistic understanding of brain function

relative to regional activations (Bullmore & Sporns 2009). Such models have shown that functional systems are sensitive to developmental/aging effects as well as exposure to substances (e.g., decreases in modularity, efficiency). The goal of this study was to determine the interaction between these two factors on graph metrics. To that end, 128 regular cannabis users (age range: 31±7 years) were scanned using resting state functional MRI (rsfMRI). After routine preprocessing of all rsfMRI images (e.g: motion correction, band-pass filtering, etc), nodes of the network (graph) were derived by registering each brain to AAL atlas yielding 45 regions in each hemisphere. The mean time series for each region was calculated by averaging the time series of all the voxels within each region. Further, a symmetric correlation matrix of size 90x90 was generated from Pearson correlation coefficients between each pair of nodes, for each subject. Each correlation matrix was further converted into a weighted network (weights being the correlation between any two nodes) and binary network (weights of the non-zero edges in the weighted graph equated to 1). Each of such binary and weighted networks was explored for two sets of network properties: global network metrics, yielding information about the topological brain properties such as small-worldness, path length, clustering coefficient, hierarchy, modularity, efficiency etc and regional nodal metrics such as nodal efficiency, betweenness, nodal efficiency, cluster and nodal participant etc. The network properties thus obtained were compared across the cannabis users to see the difference in the brain organization due to early and late onset of regular use of cannabis. Our findings suggest that effects on functional systems as measured by graph metrics are moderated by age of onset of regular use, particularly in the default mode network (DMN), fronto-parietal network (FPN; cognitive control), salience network (SN; directed attention) and subcortical (limbic/reward) network. These findings demonstrate the presence of observable effects on brain functional systems across the lifespan that are unique to the age of onset.

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777.20/14. ***Comparing effects of alcohol and marijuana: An n-back fmri study in young adults***

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Alcohol and marijuana are the most commonly used drugs during adolescence. Within the past five years, alcohol usage has declined while marijuana usage has increased. Given this increase in marijuana use among teenagers, and the potential combination of using both alcohol and marijuana during this time when brain maturation is so vital, it is critical to understand the neurophysiological impacts of both drugs on the teen brain. The optimization of brain development can be monitored by proficiency in working memory. As usage and public opinion of drug use is often a result of the perceived risk of the substance, a comparison of the performance and neurophysiological impact of each drug during a working memory task will be of considerable relevance. The purpose of the present study was to compare the effects of alcohol with those of marijuana, on a functional magnetic resonance imaging (fMRI) working memory task, in adolescent users. Methods Participants were recruited from the Ottawa Prenatal Prospective Study. Ten marijuana users were compared with 11 non-users and 11 alcohol users were compared with 11 non-users. Each participant attended one imaging session on a 1.5 T Siemens

Magnetom Symphony MR scanner for whole brain BOLD fMRI. A 2-back letter n-back task was used. For both drugs, BOLD activations during the working memory task, and performance (reaction time and errors) were compared to the respective control group of non-users using SMP8. Results/Conclusions No significant differences in performance were found between groups. fMRI analyses revealed significantly more activity in both drug groups compared to controls but the areas of increased activity during working memory were different for the 2 drugs. Alcohol users had significantly more activity in the cingulate gyrus and the right caudate nucleus, while the marijuana users engaged the middle temporal gyrus and cerebellum significantly more than controls. These findings suggest that, although both substances produce a need for additional resources to maintain successful performance, the mechanism by which they function differs. This additionally implies that, especially in the developing brain, use of both marijuana and alcohol would cause more widespread negative effects on neural processing.

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778.01/15. ***Extinguished environment elicits transient synaptic potentiation in the accumbens shell***

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Discovering mechanisms underlying inhibition of drug seeking is critical to developing new therapies for substance use disorders. Extinction training consists of animals repeatedly receiving no reinforcement for pressing a lever formerly paired with cocaine infusions. Animals successfully inhibit drug seeking when returned to the extinguished context (the operant chamber in which they underwent extinction training). This inhibition requires neuronal activity in nucleus accumbens (NA) shell and its prefrontal (infralimbic) input. Transient synaptic potentiation (tSP) in accumbens medium spiny neurons (MSNs) is a physiological correlate of behaviors requiring glutamatergic inputs into nucleus accumbens. For example, cue-induced reinstatement of drug seeking is associated with a tSP (rapid increases in AMPA:NMDA ratio and dendritic spine head diameter that normalize by the end of the reinstatement session) that requires prefrontal inputs and activation of matrix metalloproteinases (MMPs 2 and 9). As tSP is associated with behavior relying on glutamate in nucleus accumbens, and glutamate in NA shell is necessary for inhibiting drug seeking in an extinguished context, we hypothesize that exposure to an extinguished context will induce tSP in NA shell. Following 10 days of cue-paired cocaine self-administration, animals underwent 2-3 weeks of either extinction training in the same context (but without discrete cues) or home-cage abstinence. Animals were sacrificed after returning for 1 minute to the context in which they had undergone self-administration (with or without extinction training), or sacrificed without re-exposure to the context. We used whole cell patch clamp electrophysiology to examine AMPA:NMDA ratio and diIolistic labeling to image dendritic spines. Exposure to the extinguished context induced tSP in NA shell. AMPA:NMDA ratios were increased in animals re-exposed to the extinguished context relative to extinguished animals not re-exposed. Spine morphology was not affected by re-exposure to an extinguished context. No changes were noted in NA shell as a result of cocaine self-administration or extinction training alone. The fact that 1 min of exposure to the

extinguished environment increased AMPA:NMDA, but did not change spine morphology differs from tSP characterized during reinstatement in NA core, and suggests dissociable mechanisms underlying these two common measures of synaptic plasticity. Future studies will examine the role of MMPs and infralimbic inputs on tSP induced in the NAc shell by an extinguished environment.

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**778.02/16. *Elucidating the effects of atypical dopamine uptake inhibitors on the phasic release of dopamine in mice***

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Atypical dopamine uptake inhibitors, such as JHW 007, have low potential for abuse and may lead to critical discoveries in the development of treatments for psychostimulant abuse. These compounds are known to bind to the dopamine transporter with high affinity and block the pharmacological and behavioral effects of stimulant drugs of abuse, such as cocaine and methamphetamine. The objectives of this study are to explore the effects of JHW 007 and other typical and atypical dopamine uptake inhibitors on the phasic release of dopamine. We would also like to determine how these effects correlate with the previously reported behavioral and tonic dopamine level data. Fast scan cyclic voltammetry (FSCV) is an electrochemical method that allows for the study of the phasic release of dopamine both in vivo and ex vivo. In this study, phasic dopamine release in the striatum of male Swiss-Webster mice was followed both prior to, and for several hours after administration of cocaine or JHW 007 with doses ranging from 0.3 mg/kg to 32 mg/kg. This allowed us to study the effects of both drugs on the intensity, duration, and effective clearance of dopamine released during phasic events as the effects of each drug developed. Initial results indicate that both typical and atypical inhibitors effect phasic dopamine release by two mechanisms, increasing the amount of dopamine release per event, and decreasing the rate of clearance of dopamine from the intercellular space as evident by the changes in the intensity and duration of events when inhibitors are present. When cocaine is administered these mechanisms are affected in a dose dependent manner. However, atypical inhibitors such as JHW 007 do not produce the same magnitude of effect at similar doses, and may act on these two mechanisms at different times, causing the apparent shift in the dose response curve previously reported with microdialysis. In summary, our results help provide a broader view of the role of JHW 007 and other atypical dopamine uptake inhibitors in potential treatments for stimulant abuse by elucidating the mechanisms involved in the phasic release of dopamine.

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**778.03/17 *Beta-arrestin dependent regulation of cocaine self-administration in mice***

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Prolonged use of cocaine results in long lasting synaptic changes in the mesolimbic reward centers. Identification of substrates that are responsible for the induction and maintenance of such plasticity may help provide novel therapeutic targets for the treatment of substance abuse and relapse. Cocaine self-administration has been shown to abolish long term depression (LTD) in the bed nucleus of the stria terminalis (BNST), which can be rescued by blockade of the NR2B subunit of the N-Methyl-D-aspartate receptor (NMDAR). Studies have shown a similar blockade of LTD in the NAc following cocaine self-administration. We have previously shown role of the scaffolding protein,  $\beta$ -arrestin 1, in modulating GPCR expression and function by regulating pathways responsible for receptor trafficking onto the cell membrane. Therefore, we hypothesized that  $\beta$ -arrestin may be involved in regulating cocaine-induced changes in NMDAR expression and function. We trained mice lacking  $\beta$ -arrestin to self-administer cocaine, and measured synaptic activity from medium spiny neurons (MSNs) in the shell of the NAc, before and after cocaine self-administration. We found that mice lacking  $\beta$ -arrestin had increased basal AMPA to NMDA ratios in the NAc as compared to the wild-type mice. Moreover, the  $\beta$ -arrestin 1 knockout mice were slower in both acquiring, and extinguishing cocaine self-administration. Furthermore, unlike wild-type mice, the knockout mice did not show an increase in NR2B receptor expression after the acquisition of cocaine self-administration. These findings identify a, previously unknown, role of  $\beta$ -arrestin in regulating NMDAR function following cocaine self-administration.

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778.05/19. ***Cell type specific dysregulation of GABAergic plasticity in the Ventral Pallidum after extinction from cocaine self administration***

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The Ventral pallidum (VP) is the main target of the mesolimbic indirect pathway and as such integrates motivational and sensory information. It has been demonstrated that the projection from the nucleus accumbens (NAc) to the VP regulates the reinstatement of cocaine-seeking, a process which depends on normal activation of mu opioid receptors. Indeed, NAc-VP GABAergic synapses of drug-naïve animals undergo a form of presynaptic long-term depression (LTD) that is mu opioid receptor dependent. This form of plasticity is abolished in cocaine-extinguished rats due to tonic saturation of mu opioid receptors. Classically it is thought that the output from the NAc to the VP is composed exclusively of axons of medium spiny neurons (MSNs) expressing the D2 dopamine receptor (D2-MSNs). However, we have recently shown that the VP receives significant input from D1-MSNs and that projections from the NAc to VP contain D1 mRNA, questioning this strict segregation. Here we examine whether both accumbal inputs to the VP express electrically-induced LTD and the effect of extinction of cocaine-seeking behavior on each input. Using Cre dependent expression of ChR2 in either D1 or D2 MSNs we demonstrate that while both inputs exhibit LTD in drug-naïve mice, the elimination of GABAergic LTD in cocaine-extinguished mice described previously is cell type specific. While LTD can still be elicited in D1 MSN input to the VP of cocaine extinguished mice, LTD in D2 MSN terminals is abolished. This differential effect of cocaine on plasticity may be explained by differences in signaling pathways and

further research is required to identify the relevance of each of these VP afferents to the reinstatement of cocaine seeking behavior.

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**778.06/110. *Inherent individual differences in dopamine release are associated with variability in subsequent cocaine consumption***

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The mesolimbic dopamine (DA) system is heavily implicated in the onset and maintenance of cocaine use and variability within this pathway is believed to underlie individual differences in vulnerability to the development of cocaine abuse and addiction. Previous work has demonstrated that rats classified as high responders to novelty acquire cocaine self-administration more readily and are characterized by both greater DA-ergic storage capacity and cocaine-induced uptake-inhibition than those identified as low responders. Despite this evidence, the direct relationship between DA neurotransmission and individual differences in cocaine self-administration has not yet been fully elucidated. To determine the relative contributions of DA release and uptake to vulnerability to use cocaine, we measured baseline DA release and uptake dynamics in the striatum of anesthetized rats using fast scan cyclic voltammetry prior to any behavioral testing. Following recovery, the rats were provided access to cocaine-associated levers and the time to acquire, consumption of, and motivation for cocaine using fixed ratio-1 and within-subject threshold schedules of reinforcement was measured. Preliminary results suggest a strong relationship between baseline DA release and cocaine consumption while uptake did not appear to be strongly associated with any behavioral measure, potentially implicating DA reserve pools in vulnerability to cocaine use disorders. These data suggest that individual differences in cocaine self-administration may be associated with inherent variability in the mesolimbic DA system. The current findings may aid in further development of targeted pharmacotherapies to treat cocaine addiction.

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**778.07/111. *PCAF regulates the acetylation of Sigma-1 receptor chaperones***

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Sigma-1 receptors (Sig-1Rs) are endoplasmic reticulum (ER) chaperon proteins that are implicated in various neurological disorders. Sig-1Rs have received attention because of the specific involvement in drug abuse. Since antagonizing Sig-1Rs diminishes cocaine-induced behavioral responses, Sig-1Rs are expected to be potential therapeutic target of cocaine abuse. Sig-1Rs predominantly reside at the mitochondria-apposing ER subdomain (the mitochondria-associated ER membrane, MAM) but can translocate to other compartments of the cell under stimulation with cocaine. We previously showed that cocaine-induced translocation of Sig-1Rs, which intensifies the Sig-1R interaction with Kv1.2

potassium channel, plays a substantial role in behavioral and neuronal responses to cocaine in mice. Thus, the dynamic shift of the subcellular distribution is a critical step for Sig-1Rs to execute their functions at remote sites other than the MAM. However, the detailed mechanism on how the translocation is initiated is largely unknown. We found that Sig-1Rs interact with lysine acetyltransferases, p300/CBP-associated factor (PCAF) and GCN5. The interaction between Sig-1Rs and PCAF possibly occurs at the ER and the ER-Golgi intermediate compartment, and is intensified with cocaine treatment. It has been reported that acetylation of membrane proteins in the ER lumen regulates the protein translocation at the early secretory segment of the translocation pathway. We therefore hypothesized that PCAF and GCN5 may acetylate Sig-1Rs and the acetylation of Sig-1Rs may be related to the Sig-1R's translocation. We found that the acetylation level of Sig-1Rs is increased by overexpression of PCAF and decreased by knockdown of PCAF. We also found that cocaine intensifies the interaction between PCAF and Sig-1Rs. However, paradoxically, cocaine does not affect the acetylation level of Sig-1Rs. We examined whether the PCAF expression level may affect the subcellular distribution of Sig-1Rs by density gradient centrifugation, and found that overexpression or knockdown of PCAF did not significantly change the distribution pattern of Sig-1Rs. Together, our data show that Sig-1Rs are acetylated and PCAF regulates the Sig-1R acetylation level. Although cocaine affects the interaction between Sig-1Rs and PCAF, PCAF-induced acetylation of Sig-1Rs may not be the underlying mechanism whereby cocaine causes the translocation of Sig-1Rs.

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778.08/112. ***Synapse-specific deconstruction of endocannabinoid signaling in the nucleus accumbens shell***

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Aberrant glutamatergic transmission within the nucleus accumbens shell (NAcSh) is heavily implicated in the development and reinstatement of drug-induced addiction-like behaviors. Integration of glutamatergic input from the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) by NAcSh medium spiny neurons is thought to encode stimulus salience and direct associative learning processes. Moreover, long-lasting adaptations at these synapses have been repeatedly linked to drug self-administration and the incubation of drug craving in rodent models of addiction. Extensive investigation has highlighted NAcSh post-synaptic plasticity mechanisms as highly-penetrant molecular mediators of maladaptive associative-learning pathologies. However, less is known regarding presynaptic regulatory mechanisms and their contribution to drug-induced remodeling of reward circuitry function. Presynaptic regulation in the NAc via endocannabinoids (eCBs) and the cannabinoid type-1 receptors have been correlated with non-contingent drug exposure and self-administration behaviors. However, how the eCB system functions at discrete synapses remains unknown. Here, we utilize whole-cell electrophysiology, transgenic D1tdTom marker mice, pharmacology, and optogenetics to examine how the eCB system controls glutamatergic input onto NAcSh medium spiny neurons. We have found that eCB plasticity

induced by low-frequency stimulation is cell-type specific. Future studies will examine how this plasticity is affected by psychostimulant exposure and abstinence with the aim of developing novel addiction therapeutics.

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778.09/113. ***Cocaine and stress disrupt mGluR/sK inhibition on dopamine neurons of the VTA***

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Both environmental stressors and drugs of abuse cause enduring cellular adaptations in the ventral tegmental area (VTA) which contribute to addiction. In rats with a history of cocaine use, stressors promote relapse of drug seeking by enhancing corticotrophin-releasing factor (CRF) signaling in the VTA. One potential mechanism by which stress and drugs of abuse overlap is the induction of CRF-dependent plasticity at glutamatergic synapses. The interaction between stress, drugs of abuse, CRF, and glutamate in the VTA, however, remains poorly understood. Aside from promoting excitatory signaling via ionotropic receptors, glutamate also recruits inhibition in VTA neurons via the activation of postsynaptic metabotropic glutamate receptors (mGluRs), mGluRs mobilize intracellular calcium stores to activate inhibitory sK channels. This Ca<sup>2+</sup>-dependent signaling is potentiated by CRF. Here, using whole cell patch clamp electrophysiology recordings from VTA neurons, we investigated mGluR - sK channel inhibition after single and repeated exposure to the ecologically valid stressor TMT (a component of fox odor), or repeated exposure to cocaine. Single TMT exposure facilitated mGluR - sK currents, an adaption normalized by either the CRF receptor 1 (CRF-R1) antagonist CP-156254 or the CRF receptor (CRF-R2) antagonist K41498. However, repeated TMT exposure weakened the evoked mGluR - sK current, a condition that was unabated by blockade of CRF-R2. In addition, repeated TMT exposure significantly increased the frequency of spontaneous miniature outward currents (SMOCs). These SMOCs were observed prior to synaptic stimulation, persisted in the presences of TTX, and were blocked by either depletion of intracellular calcium stores with CPA, or by the irreversible sK channel blocker apamin. Activation of sK channels may be enhance due to dysregulated calcium leak from intracellular stores. Interestingly, repeated administration of cocaine (IP) with 7-14 days of withdrawal resulted in similar increases in SMOCs. We propose that cocaine as well as stress, alters mGluR inhibition in DA neurons through impairment of intracellular Ca<sup>2+</sup> signaling.

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778.10/114. ***Cocaine mediated molecular regulation of mitochondrial dynamics in nucleus accumbens projection neuron subtypes***

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Altered brain energy homeostasis is a hallmark adaptation occurring in the cocaine-addicted brain. This includes alterations in glucose metabolism, glutamate homeostasis, and oxidative stress. Recent studies demonstrate that mitochondria dysfunction is associated with psychiatric disorders. However, mitochondrial dynamics have not been thoroughly addressed in cocaine abuse. Our data demonstrate that genes important for mitochondria biogenesis and function are upregulated in nucleus accumbens (NAc) of rodents that self-administer cocaine (FR1 schedule, 1mg/kg/infusion) and in postmortem NAc of cocaine dependent individuals. We next examined mitochondrial biogenesis and function genes in the two NAc projection medium spiny neuron (MSN) subtypes, those enriched in dopamine D1 vs. D2 receptors. Using the RiboTag methodology, we observe an up-regulation of ribosome-associated mRNA of many mitochondrial biogenesis and function genes in D1-MSNs but a decrease in D2-MSNs after repeated cocaine (7 days, 20 mg/kg). We have generated a Cre inducible adeno-associated virus (AAV)-double inverted floxed open reading frame (DIO)-mito-dsRed to label mitochondria in D1-MSNs and D2-MSNs using D1-Cre and D2-Cre mouse lines. This will allow us to examine mitochondrial volume and number in MSN subtypes after repeated cocaine exposure. Additionally, we have developed an AAV-DIO to overexpress peroxisome proliferator-activated receptor-gamma coactivator-1 $\alpha$  (Pgc1 $\alpha$ ), a transcriptional coactivator of mitochondrial biogenesis and function genes. Overexpression of Pgc1 $\alpha$  in NAc D1-MSNs enhanced cocaine conditioned place preference and cocaine-induced locomotion, while Pgc1 $\alpha$  expression in D2-MSNs reduced these behaviors. Another gene we are pursuing is dynamin-related protein 1 (Drp1), a GTPase that directly binds to the outer mitochondrial membrane to promote mitochondria division hence it plays an important role in generating new mitochondria. We find that the active form of Drp1 protein is increased in NAc and the Drp1 gene is increased in D1-MSNs but reduced in D2-MSNs after repeated cocaine (7 days, 20mg/kg). We are developing Cre-inducible AAVs for wildtype, constitutively active, and a dominant-negative Drp1 so we can test Drp1 function in MSN subtypes in cocaine-related behaviors. Collectively, our findings demonstrate altered molecular mechanisms governing mitochondrial dynamics in the two MSN subtypes with cocaine exposure.

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778.11/115. ***Cocaine self-administration and cue-reinstatement disrupt Kv7 (KCNQ) channel inhibition in the prefrontal cortex***

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In human cocaine addicts, re-exposure to drug-paired cues induces hyperactivity in the prefrontal cortex (PFC) that precipitates drug-craving and relapse. While the underlying mechanisms are unknown, they likely involve cellular adaptations in intrinsic ion channel signaling. For example, pyramidal cells in the PFC normally display robust spike-frequency adaptation (accommodation) that limits repetitive neuronal firing. This firing pattern is mediated in part by Kv7 (KCNQ) K<sup>+</sup> channels, which are activate at

subthreshold potentials, are non-inactivating, and are sensitive to inhibition by multiple neuromodulators known to be released in response to cues. Therefore, using whole cell patch clamp electrophysiology we recorded from L5 pyramidal cells in the prelimbic (PL) PFC of rats after a history of chronic cocaine self-administration and extinction training, with or without re-exposure to cocaine-paired cues and investigated Kv7 channel function. After cocaine self-administration and extinction, cells demonstrated (a priori) hyperexcitable firing rates, loss of spike accommodation, and reduced Kv7 channel inhibition. These adaptations could be normalized by acute blockade of dopamine D1 receptors, inhibition of PKA, depletion of intracellular Ca<sup>2+</sup>, or stabilization of Kv7 ion channels directly with retigabine. This suggests that excessive dopamine D1 receptor signaling disrupts Kv7 channel function. Re-exposing rats to cocaine-paired cues for 30min further enhanced neuronal excitability, involving calcium-store dependent desensitization of Kv7 channel activity. These cellular adaptations may contribute to cue-induced drug seeking, since in vivo infusion of retigabine into the PFC prior to cue-induced reinstatement testing blocked cocaine-seeking behavior. Taken together these data suggest that chronic cocaine experience enhances cue-induced PFC excitability by disrupting intrinsic Kv7 channel mediated spike accommodation, resulting in repetitive neuronal firing in response to depolarizing (excitatory) inputs. This neuroadaptation may underlie the enhanced saliency of drug-related cues that trigger relapse in cocaine addicts.

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778.12/116. ***Dynamic changes in nucleus accumbens miR-495 expression across cocaine self-administration and reinstatement***

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We previously found that the microRNA miR-495 is highly expressed in the striatum, targets several addiction-related genes (ARGs), and is downregulated in the nucleus accumbens (NAc) 1 h following both acute and sensitizing cocaine injection regimens. We then found that miR-495 overexpression in the NAc shell (NAcSh) decreases ARG expression, cocaine self-administration (SA) under progressive ratio schedule of reinforcement, and responding during extinction and reinstatement. These findings suggest miR-495 regulates genes that are involved in motivation for cocaine. Here, we sought to characterize changes in NAc miR-495 expression across different lengths of cocaine SA, and following cue and cocaine-primed reinstatement. Adult, male Sprague-Dawley rats were trained to lever press for cocaine (1.0 mg/kg/0.1ml, IV) under a variable ratio (VR) 5 schedule of reinforcement. Control rats received yoked saline infusions. Rats were sacrificed 1 h following either the 1st (i.e., Day 1 group) or the 22nd SA session that either followed 1- or 21-d abstinence period (i.e., Day 22 or Relapse group, respectively). Total RNA was isolated from NAc tissue and miR-495 expression was measured using qRT-PCR. We found that miR-495 levels were significantly increased in the Day 1 and Day 2 SA groups in the NAcSh compared to saline controls, but no change was found in the NAcSh of the Relapse group or

between any groups in the NAc core. A separate group of rats underwent SA training (0.75 mg/kg/0.1ml, IV; VR5), followed by daily extinction sessions, and then were either tested for cue or cocaine-primed reinstatement. For cocaine-primed reinstatement, cocaine- and saline-trained rats received either a cocaine (15 mg/kg, IP) or saline injection on test day. Rats were sacrificed immediately following the 90-min test session. We found no change in NAc miR-495 expression following cue reinstatement, but did find a significant decrease following a cocaine-primed test day, regardless of whether rats were trained with cocaine or saline. This latter effect is consistent with our previous findings where experimenter-delivered cocaine downregulates NAc miR-495 expression. In contrast, self-administered cocaine upregulates miR-495 levels, specifically in the NAcSh. This effect only occurs during SA acquisition and maintenance, suggesting miR-495 increases in the early stages of acquiring cocaine abuse-related behavior, but not in later stages. Given that miR-495 targets several ARGs and increasing its expression in the NAcSh suppresses motivation for cocaine, the blunted increase in miR-495 during relapse may contribute to neuroadaptations underlying cocaine dependence.

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**778.13/117. *The neuronal RNA-binding protein HuD interacts with Argonaute proteins and GW182 proteins in an RNA-dependent manner relieving miRNA-mediated repression***

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The RNA stability factor HuD is an RNA-binding protein that binds specific U-rich sequences in the 3'UTR of target mRNAs. This interaction serves to stabilize mRNAs, allowing for increased translation of the targets. MicroRNAs (miRNAs) also bind specific sequences in the 3'UTR of target mRNAs, but unlike HuD, this interaction most often results in decreased translation through silencing or degradation. MiRNAs function in the context of the RNA-induced silencing complex (RISC), which includes Argonaute (Ago) proteins. Ago proteins interact with GW182 proteins, which are required for miRNA-mediated gene silencing. GW182 proteins accumulate in GW-bodies, also known as P-bodies, which are sites for mRNA degradation. mRNAs may also be stored in P-bodies, in the cell body as well as in neuronal processes, until they are needed. Previously, we reported that HuD and miR-495 have many target mRNAs in common, as the miR-495 seed sequence is complementary to the HuD GU-rich binding motif and that many of these common targets are implicated in drug addiction (Gardiner et al. 2013; SfN Poster 350.10). Thus, HuD and miR-495 compete for the binding and regulation of addiction-related genes such as BDNF and CAMK2a, which are enriched in shared binding-sites in their 3'UTRs. Interestingly, when HuD was transfected into HeLa cells that were previously subjected to miR-495-mediated repression, reporter constructs showed robust derepression. However, the opposite did not occur when miR-495 was added to cells previously treated with HuD. Here we show that in the mouse striatum, a region important for addiction-related processes, HuD interacts with Ago proteins and that this interaction is in part RNA-mediated. We also show that HuD interacts with the P-body marker GW182. In addition, HuD colocalizes with GW182 in neuroblastoma cells treated with hydrogen peroxide. These findings suggest a mechanism by which HuD may "rescue" mRNAs from miRNA-mediated repression in P-bodies, allowing

for increased translation. Furthermore, since HuD is expressed in the nucleus accumbens and regulated by cocaine, HuD-mediated target derepression may be involved in the post-transcriptional control of gene expression during the establishment of addiction-related behaviors.

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778.14/118. ***Neuronal RNA-binding protein hud regulates addiction-related target mRNAs, structural plasticity, and cocaine addiction-related behaviors***

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Post-transcriptional mechanisms play an important role in nervous system development and function, however, very little is known about their role in the etiology of substance use disorders. RNA binding proteins (RBPs) provide one such regulatory mechanism in post-transcriptional regulation. HuD is a neuronal specific RBP that associates with the 3'UTR of specific mRNAs containing AU-rich instability elements (AREs). Through association with these regions, HuD stabilizes mRNA transcripts protecting them from degradation. We found that HuD targets include those that have been implicated in addiction, including CaMKIIa, Bdnf, Mef2c, and Arc. Additionally, HuD (ELAVL4) itself was found within the KARG suggesting it may play a role in this disorder. Using a dual luciferase expression system, we demonstrated that the Camk2a, Arc, and the long and short 3' UTR variants of BDNF transcripts (Bdnf-L, Bdnf-S) are direct targets of HuD. Confirming this, we found that mice overexpressing neuronal specific HuD (HuD OE; Camk2a-myc-ELAVL4/Npb; Bolognani et al., 2006) had increased mRNA expression of targets such as CaMKII $\alpha$  and Bdnf, especially within the Nucleus Accumbens (NAc). Increased protein expression of these targets was also found within this region. Since many HuD targets have roles in structural plasticity of neurons, specifically within the NAc, we were interested in the role that HuD may play in this phenomenon. We found that HuD OE animals had increased percentage of immature thin spines, with a decrease in intermediate stubby spines within NAc neurons. However, there were no differences in the percentage of mature mushroom spines. Next, we were interested in the translation of these molecular and structural alterations to behavior. We found that HuD OE mice are more sensitive to the acute locomotor stimulatory actions of 7.5 mg/kg cocaine compared to wild type (WT) littermates. Although their initial response to cocaine was elevated, the animals did not exhibit sensitization to cocaine suggesting a ceiling effect of drug-induced locomotor activity. In conditioned place preference model, we found that HuD OE spend more time in the cocaine (15 mg/kg) associated chamber compared to WT littermates. Finally, this effect may be limited to drug associated cues, as HuD OE and WT animals did not show a difference in their acquisition or extinction of appetitive behavior. However, upon exposure food-paired cues, HuD OE animals show increased reinstatement behavior. Together, these results suggest that HuD may play a role in addiction-related alterations in gene expression, plasticity, and behavior.

**778.16/120. Phasic neuronal activity in the anterior cingulate cortex robustly differentiates water and cocaine cues in rhesus macaque monkeys**

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Prior studies in rodents suggest that cues associated with natural rewards, such as food and water, are represented by phasic activity in a common, overlapping subpopulation of neurons in the ventral striatum. Conversely, phasic cocaine cue encoding has been shown to manifest in a parallel, largely non-overlapping ensemble of neurons in that region. Though these electrophysiological studies have substantiated parallel processing of motivationally salient cues in the rodent ventral striatum, the topography of encoding of drug vs non-drug reward is less well characterized in the primate brain. Rodent functional imaging data implicate a broad tapestry of brain reward circuitry in the differentiation of cocaine and natural reward cues, including the dorsal striatum and medial prefrontal cortex. In primates, both orbitofrontal and anterior cingulate cortex have been shown to selectively encode distinct non-drug rewards. The purpose of the current study was to extend electrophysiological differentiation of cocaine versus non-drug reward associated cues to a broader neuroanatomical framework in the non-human primate brain. We simultaneously recorded multiple single units in the ventral and dorsal striatum, as well as the anterior cingulate and orbitofrontal cortices of rhesus monkeys during both water and cocaine self-administration blocks within the same sessions. We identified differential encoding of water and cocaine cues across all brain regions. However, we found that neurons in the anterior cingulate cortex distinguish cocaine from water cue conditions more robustly than neurons in the other regions of interest. These data implicate the anterior cingulate cortex as a structure of particular importance in the differentiation of drug and non-drug reward.

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**778.20/124. Enhanced sensitivity to repeated cocaine increases perineuronal net staining in the adult rat medial prefrontal cortex**

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Perineuronal nets (PNNs) are unique structures of extracellular matrix in the central nervous system. Within the medial prefrontal cortex, PNNs surround the soma and proximal dendrites of parvalbumin (PV)-containing, GABAergic interneurons. The appearance of PNNs during development coincides with the closure of critical periods, during which time external stimuli influence development of neuronal circuits. Cocaine is a psychomotor stimulant that increases locomotor activity in rats; after repeated cocaine exposure, rats become sensitized to cocaine and display an enhanced locomotor response. We

sought to determine the effect of cocaine exposure on PNNs within the medial prefrontal cortex. We hypothesized that exposure to a strong external stimulus (cocaine) during adulthood would increase the intensity of PNNs within the prefrontal cortex. To test this, we exposed adult, male rats to 1 or 5 days of cocaine (15 mg/kg, i.p.) and locomotor activity was measured each day to assess sensitization. Two hours following the last injection, rats were sacrificed and the prefrontal cortex was assessed for PNN intensity. Preliminary results suggest that 2 hr following the last cocaine injection, PNN intensity was increased in the prelimbic region of the prefrontal cortex but was unaffected in the infralimbic or medial orbitofrontal regions. This increased intensity was positively correlated with sensitized locomotor activity, suggesting that PNN intensity in the prelimbic region may serve as a functional read-out of cocaine-induced motivational behavior. In addition to PNNs, we also analyzed PV expression. These results demonstrate that exposure to cocaine increases PNN intensity within the prelimbic region of the prefrontal cortex and suggest that repeated cocaine may render the medial prefrontal cortex resistant to normal physiological stimuli.

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778.21/125. ***Emergence of endocytosis-dependent mglur1-ltd at nucleus accumbens synapses during withdrawal from cocaine self-administration***

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Extended-access cocaine self-administration induces a progressive enhancement of cocaine craving during withdrawal termed “incubation of craving”. Rats evaluated after >1 month of withdrawal (“incubated rats”) display alterations in signaling at medium spiny neuron (MSN) synapses of the nucleus accumbens (NAc), including elevated levels of Ca<sup>2+</sup>-permeable AMPA receptors (CP-AMPA) and a transition in group I metabotropic glutamate receptor (mGluR)-mediated synaptic depression from mGluR5- to mGluR1-dependent. To determine the time-course over which these adaptations appear and further characterize the emergent form of mGluR1-mediated synaptic depression, we conducted whole-cell patch-clamp recordings in NAc core MSN of “incubated rats” at multiple time-points during withdrawal. Elevated synaptic contributions from CP-AMPA, as well as the loss of mGluR5- and gain of mGluR1-mediated synaptic depression, were not detected between 7 and 25 days of withdrawal, but were present following at least 35 days. Furthermore, the previously identified mGluR1-mediated suppression of the EPSC was found to be a form of long-term depression (mGluR1-LTD). We also investigated the mechanism underlying this mGluR1-LTD by conducting recordings in the presence of dynamin inhibitory peptide or pep2-EVKI, which disrupt endocytosis and PICK1 regulation of calcium-impermeable AMPAR (CI-AMPA) trafficking, respectively. Together, our results indicate that mGluR1-LTD involves a non-obligatory swap of CP-AMPA and CI-AMPA mediated by dynamin-dependent internalization and PICK1-dependent insertion. Together, these results elucidate the time-course for the emergence of multiple adaptations during withdrawal, in addition to the mechanisms underlying mGluR1-LTD at NAc MSN synapses after the “incubation” of cocaine-craving.

779.01/126. **Effect of naltrexone on neural response to risky decision making**

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Introduction: Persons who use methamphetamines (MA) often engage in risky decision-making. Naltrexone blocks opioid mu receptors in the reward pathway that may modulate impulsivity. We assessed the effect of naltrexone on impulsivity and its neural correlates in MA dependence. Methods: We evaluated 42 MA-dependent participants on a probability-delay discounting (PDD) fMRI task before and 4 weeks after randomization to extended-release naltrexone (XR-NTX 380mg) or placebo injection. The PDD asks participants to choose between an immediate, certain monetary reward and alternative reward of varying magnitude, time to receipt, and odds against winning. We calculated area under the curve (AUC) for delay and probability. AFNI amplitude modulated regression identified brain regions whose BOLD response scaled with the size of the reward characteristic. Linear mixed models assessed within subject (time) by group main effects and interaction (voxel threshold  $p=0.01$ ; cluster threshold familywise  $\alpha<0.05$ ). Results: Participants were similar by treatment group (Table). Performance on the PDD did not differ between groups over time, but probability AUC decreased for XR-NTX group, suggesting decreased impulsivity. fMRI analysis found significant treatment by time interaction clusters for probability and delay (Figure). Cluster voxels were less responsive to stimulus in the NTX group, but unchanged for placebo.

Table: Comparison of demographic and discounting measures between groups before and after XR-NTX injection.

Demographics												
	Age (SD)		Male		Education (SD)		pre	post	pre	post		
XR-NTX (19)	39.3	10.6	76.0	12.6	2.04	0.61	0.25	0.61	0.24	0.36	0.25	
	0.31	0.26										
Placebo (23)	36.7	9.53	70.4	12.5	0.77	0.47	0.24	0.50	0.21	0.34	0.19	
	0.26	0.12										

\*Significant main effect of visit ( $F(1,40) = 5.7, p = 0.021$ ).

Conclusions: In this sample, XR-NTX decreased sensitivity to delay and probability in behaviorally relevant brain regions. The PDD may be useful for studying the neural basis of behavioral response to other substance use disorder treatments.

779.05/130. ***Changes in cortico-striatal neuroplasticity following methamphetamine***

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Methamphetamine (meth) abuse induces changes in the prefrontal cortex (PFC) and nucleus accumbens (NAc), which exert inhibitory control over maladaptive behaviors like drug over-consumption and mediates reward-related behaviors, respectively. Hence, meth-induced functional impairment of these two regions may increase abuse vulnerability. Here, we describe changes in cortico-striatal neuroplasticity following a contingent and non-contingent meth administration protocol. In Experiment 1, rats were given non-contingent i.p. injections of 4 mg/kg meth or saline at 2 hr intervals in one day. Rats were killed 7 days later and whole-cell patch clamp recordings were performed in the dorsomedial PFC (dmPFC) and field potentials in the NAc core (NAcc) of the same animals. In the dmPFC, meth increased the AMPA/NMDA ratio in meth groups compared to controls indicating potentiated state of deep-layer PFC pyramidal neurons. In the NAcc we observed a significant paired pulse depression (indicating a higher neurotransmitter release probability) in meth group when compared to controls. Also, input/output curves revealed an overall decrease in synaptic strength of meth rats. In Experiment 2, rats received one-hour meth self-administration (SA) sessions for 5 days, followed by 10 days of six-hour long access sessions and yoked saline controls. Meth was delivered IV on an FR1 schedule at a volume of 20 µg/50 µl infusion and rats were killed 7 days after discontinuation of meth. In the dmPFC, AMPA/NMDA ratio was unaffected in meth SA rats when compared to controls although, only n=2 rats have been recorded so far. In the NAcc, we assessed long term depression (LTD) and potentiation (LTP) to understand the synaptic strength using low frequency (5Hz, 900 times) and high frequency (100Hz, 4 times) stimulation protocols. Meth SA caused a loss of LTP and significantly lessened the degree of LTD induction compared to the controls. Combined, our findings indicate changes in synaptic neurotransmission in both the dmPFC and NAcc following contingent and non-contingent meth. The relationship between meth pharmacology alone or combined with behavior output has yet to be defined. Additional questions need to determine how these maladaptive consequences impact glutamate release, reuptake, and relapse to meth-seeking behavior.

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779.09/134. ***Genome-wide DNA hydroxymethylation patterns in the rat nucleus accumbens consequent to methamphetamine self-administration***

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In animal models of methamphetamine addiction, rats learn to self-administer the drug and accelerate their intake over time. However, these self-administration (SA) models do not include adverse

psychosocial consequences that are embodied in the diagnostic criteria of substance use disorders. Indeed, adverse outcomes are relevant factors that can promote abstinence in humans. Here, we studied the genome-wide DNA hydroxymethylation consequences of suppression of drug seeking by repeated foot-shocks (punishment). Rats were trained to self-administer methamphetamine for 1 h per day for 20 days. Reward delivery was paired with a tone-light cue sequence. Subsequently, 50% of the lever-presses were punished by mild foot-shock for 10 days for one methamphetamine SA group whereas lever-presses were not punished for another methamphetamine SA group. Shock intensity (0.18-0.36 mA, 0.5 sec) was gradually increased over time. Response-contingent punishment suppressed extended-access methamphetamine taking in some rats (Punishment-sensitive, PS) whereas other rats were punishment-resistant (PR) and continued to press for methamphetamine at a high level. Rats were euthanized at 24 hours after the last self-administration session and brain regions were collected for further molecular experiments. Tissues from the nucleus accumbens (NAc) were used to identify differentially hydroxymethylated regions by using hydroxymethylated DNA immunoprecipitation followed by whole genome sequencing (hMeDIP-Seq). Our study identified several differentially hydroxymethylated regions in the NAc of methamphetamine self-administering rats. Several classes of genes showed differential DNA hydroxymethylation in rats that showed the PR compared to the PS phenotype. These included genes involved in epigenetic regulation (DNA methyl-transferase1-associated protein 1 and Sin3A), genes that code for protein phosphatases (protein phosphatase 1, regulatory subunit 12A), and several microRNAs (miRNA 146a). In comparison to the PS group, the PR phenotype also showed increased DNA hydroxymethylation at long interspersed nuclear elements (LINE), with these differences being potentially responsible for genomic instability and consequent altered gene expression in the PR rats. These observations are consistent with our previous suggestions that methamphetamine SA is associated with diverse molecular modifications in the brain. Our findings support the idea of developing specific epigenetic agents in order to expand the therapeutic armamentarium against methamphetamine addiction.

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779.10/135. ***Efficacy of a tetanus-toxoid succinyl-methamphetamine vaccine differs between male and female mice***

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Background: We previously reported that the behavioral effects of cocaine and response to an anti-cocaine vaccine significantly differed between male and female mice. Here we assessed potential sex differences in response to methamphetamine (MA). We also determined whether the efficacy of a vaccine targeting MA would show sex-dependent effects. The anti-MA vaccine was constructed of tetanus-toxoid linked to succinyl-methamphetamine (TT-SMA), absorbed with aluminum hydroxide, and

administered with the adjuvant E6020, Toll-like receptor-4 agonist. Methods: Locomotor activity was compared between male and female mice (BALB/c) following administration of various doses of MA (1-4mg/kg) in 90-min sessions. Separate groups of male and female mice, vaccinated with TT-SMA and boosted 3-weeks later, were employed to determine plasma anti-MA antibody concentrations at several time points across a 12-week period. Vaccinated and non-vaccinated female mice were administered MA (2.0mg/kg) and locomotor activity assessed. Brain levels were also evaluated in vaccinated and non-vaccinated mice 30-min following MA administration to assess whether the vaccine effectively decreased the ability of MA to cross the blood-brain barrier. Results: MA elicited greater locomotor activity in female compared to male mice at all doses tested ( $p's < 0.05$ ). Following vaccination, antibody levels increased after a boost at week 3 in both male and female mice; however, the increase was greater in female mice ( $p < 0.05$ ). Female mice vaccinated with SMA-TT showed attenuated MA-induced locomotor activation (2.0mg/kg;  $p < 0.05$ ) and significantly lower brain levels of MA compared to non-vaccinated female mice ( $p < 0.001$ ). Conclusions: Results suggest female mice are more sensitive to the locomotor activating effects of MA and to the immunogenicity of the TT-SMA vaccine. That the vaccine attenuated MA-induced locomotor activation is likely due to slowing MA brain penetration and supports the future development of this vaccine construct for the treatment of MA addiction, particularly in female addicts.

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779.11/136. ***Methamphetamine reward: contribution of toll-like receptor 4 and proinflammatory mediators***

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Drug reward has long been attributed to neuronal responses, chiefly within the mesolimbic dopamine (DA) pathway. Recent evidence suggests that glial activation within the brain is also required for drug reward. Morphine and cocaine activate glial Toll-Like Receptor 4 (TLR4), resulting in proinflammatory, neuroexcitatory signaling. TLR4 is an innate immune pattern recognition receptor, expressed principally in microglia in the CNS. TLR4 appears to recognize morphine and cocaine as xenobiotics (i.e., substances foreign to the brain), triggering CNS glial signaling, akin to the immune responses elicited by bacteria. The ensuing proinflammatory cascade can have neuroexcitatory consequences. In the case of morphine or cocaine, blockade of TLR4; 1) prevents induction of CNS immune activation, 2) suppresses conditioned place preference (CPP), 3) blocks drug-induced DA increases within the nucleus accumbens (NAc) and 4) attenuates drug self administration. These findings indicate that drug-induced proinflammatory CNS glial signaling is necessary for drug reward and reinforcement. Methamphetamine (METH) is thought to exert its rewarding effects via reversal of DA transporters in the mesolimbic DA pathway. Repeated METH use is also associated with neurotoxicity. There are indications that CNS glial

activation may contribute to METH's rewarding and neurotoxic effects. However, the mechanism by which METH triggers a CNS glial response is unknown. Here, we show that METH can bind to TLR4 to initiate glial reactivity. Systemic METH TLR4-dependently induces upregulation of proinflammatory markers in the brain, notably within the ventral tegmental area (VTA). Systemic TLR4 antagonism also attenuates METH CPP and METH-induced increases of DA within the NAc. We recently demonstrated that the rewarding effects of cocaine are dependent on IL1 $\beta$  signaling within the VTA. METH also initiates CNS glial responses within the VTA. However, intra-VTA mRNA is upregulated for IL6 but not IL1 $\beta$  at the timepoints tested. Studies are currently underway to investigate whether METH-induced NAc DA increases and METH self-administration are dependent on intra-VTA IL6 signaling. Interestingly, IL6 has been linked to METH neurotoxicity, suggesting a role for METH-induced CNS glial signaling underlying both reinforcement and neurotoxicity. Our data suggest that METH-activation of TLR4 contributes to its rewarding effects, as well as implicating a possible mechanism underlying neurotoxicity. These findings provide further support for the xenobiotic hypothesis, and indicate that TLR4 may be a promising target for pharmacological intervention to treat drug abuse.

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779.12/137. ***Alpha-synuclein elevation shapes dopamine neurotransmission via a DAT dependent mechanism***

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Alpha-synuclein is a small cytosolic protein enriched in the presynaptic terminals. Although its physiological function remains unknown, alpha-synuclein has been implicated in a number of pathological conditions coined synucleinopathies, which include Parkinson's disease. Though, the neuroprotective and/or neurotoxic effects of endogenous levels of alpha-synuclein is debated, it is clear that pathological levels of alpha-synuclein negatively affects the activity of dopaminergic neurons, albeit with less understood mechanism. Here we examined whether overexpression of wild-type alpha-synuclein influences the activity of dopamine neurons by measuring the spontaneous firing activity and action potential morphology of dopamine neurons. Electrophysiological results revealed that alpha-synuclein elevation decreases the frequency of spontaneous firing of dopamine neurons, increases action potential half-width and reduces afterhyperpolarization (AHP) magnitude. Alpha-synuclein overexpression enhances the action potential half-amplitude following amphetamine (AMPH) exposure compared to neurons expressing physiological levels of alpha-synuclein. We used GCaMP6, a highly sensitive calcium sensor, to study calcium dynamics in the neuronal cell bodies and processes when alpha-synuclein is overexpressed. In the absence of stimulation, as compared to control neurons containing endogenous alpha-synuclein levels, alpha-synuclein over-expression increased calcium spike amplitude and decreased spike frequency in neuronal cell bodies, but not in the neuronal process. Since activation of dopamine transporter (DAT) has been shown to increase intracellular calcium, we asked whether alpha-synuclein regulation of intracellular calcium is DAT dependent. We found alpha-synuclein

regulation of calcium mobilization in the cell body and neuronal processes was blocked by inhibition of dopamine transporter; whereas, amphetamine activation of DAT further enhanced the calcium spike amplitude in both the neuronal cell bodies and processes that overexpressed alpha-synuclein. These preliminary data suggest alpha-synuclein elevation uniquely impacts dopamine neurotransmission via a DAT-dependent mechanism.

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**779.13/138. *Preferential enhancement of MDMA-induced accumbens 5-HT over DA levels is amplified by 5-HT<sub>2C</sub> receptor antagonism***

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Actions of serotonin (5-HT) and dopamine (DA) systems underlie unique behavioral and neurochemical responses induced by 3, 4-methylenedioxymethamphetamine (MDMA or "Ecstasy"). 5-HT receptors can modulate 5-HT and DA function, but their specific role in MDMA-induced effects are yet to be determined. This study was conducted to investigate the effects of 5-HT<sub>2c</sub> receptor antagonism on MDMA-induced changes in nucleus accumbens (NAcc) extracellular 5-HT and DA levels, locomotor activity and spontaneous behaviors. During 2-h in vivo microdialysis test session, MDMA-naïve rats were treated with either saline (0.2 ml) or the 5-HT<sub>2C</sub> selective antagonist, SB 242084 (1.0 mg/kg, i.v.) 30-min prior to a self-administered infusion of MDMA (3.0 mg/kg, i.v.) or saline (0.1 ml). Saline pretreated rats showed pronounced increases in NAcc extracellular 5-HT (approx 5-fold) and DA (approx 2-fold) after MDMA administration. Pretreatment with SB 242084 resulted in significantly enhanced extracellular NAcc 5-HT (approx 9.5-fold) and behaviors associated with serotonin syndrome (e.g., low body posture, forelimb and hindlimb treading) after MDMA injection. MDMA-stimulated NAcc DA and locomotor activity was comparable in SB 242084 and saline pretreated animals and SB 242084 alone did not alter NAcc 5-HT, DA or behavioral activities prior to MDMA administration or after saline injection. Our findings indicate that 5HT<sub>2C</sub> receptor antagonism in combination with MDMA preferentially amplifies MDMA-induced NAcc 5-HT neuronal and behavioral responses compared to NAcc DA and DA-mediated effects.

**779.14/139. *PKC- $\beta$  inhibitors attenuate amphetamine and cocaine stimulated dopamine release***

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Amphetamine abuse afflicts over 13 million people, and there is currently no universally accepted treatment for amphetamine addiction. Amphetamine serves as substrate for the dopamine transporter (DAT) and reverses the transporter to cause dopamine efflux in addition to inhibiting the vesicular monoamine transporter (VMAT) to promote dopamine exocytosis. Activation of protein kinase C enhances extracellular dopamine in the presence of amphetamine by enhancing the reverse transport of dopamine and internalizing the D<sub>2</sub> autoreceptor. We previously demonstrated that PKC $\beta$

inhibitors block amphetamine-stimulated dopamine efflux in rat striatum in vitro. In this study, we utilized in vivo microdialysis in live, behaving rats to assess the effect of the PKC $\beta$  inhibitors enzastaurin and ruboxistaurin on amphetamine-stimulated increases in monoamines and their metabolites. A 30 min perfusion of the nucleus accumbens core with 1  $\mu$ M enzastaurin or 1  $\mu$ M ruboxistaurin reduced amphetamine-stimulated efflux of dopamine and its metabolite 3-methoxytyramine by approximately 50%. The inhibitors also significantly reduced extracellular levels of norepinephrine and its metabolite normetanephrine after amphetamine. The stimulation of locomotor behavior by amphetamine, measured simultaneously with the analytes, was comparably reduced by the PKC $\beta$  inhibitors. Ruboxistaurin also attenuated cocaine stimulated extracellular dopamine, a process that would not be dependent upon DAT reversal. In order to see if this process was D2 autoreceptor mediated, we examined the effect of ruboxistaurin on cocaine activation when D2 receptors were blocked with raclopride. The inhibitory effect of ruboxistaurin was reduced in the presence of cocaine and raclopride, suggesting that ruboxistaurin action involved D2 autoreceptors. Using a stable isotope label retrodialysis procedure, we determined that ruboxistaurin had no effect on basal levels of dopamine, norepinephrine, glutamate, or GABA. Our results support the utility of using PKC $\beta$  inhibitors to reduce the effects of amphetamine and cocaine.

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779.15/140. ***Amphetamine sensitization requires dopamine neuron glutamate cotransmission***

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Increased activity of dopamine (DA) neurons projecting to the nucleus accumbens (NAc) shell is thought to mediate the initial reinforcing effects of abused drugs such as amphetamine (Covey et al., TINS, 2014). Mesoaccumbal DA neurons are distinguished by showing the highest incidence of glutamate (GLU) cotransmission; however it is not clear whether DA neuron GLU cotransmission is involved in the development of drug-induced responses. Transgenic approaches have abrogated GLU cotransmission by conditional knock out of the vesicular GLU transporter VGLUT2 in DA neurons; VGLUT2 cKO mice show a reduced response to amphetamine, but this could be due to reduced DA or GLU release (Birgner et al., PNAS, 2010), as VGLUT2 cKO mice also have reduced DA neuron numbers, DA content and release (Hnasko et al., Neuron, 2010; Fortin et al., JNS, 2012). To address the role of DA neuron GLU cotransmission specifically, we targeted GLS1, which encodes the GLU synthetic enzyme glutaminase that mediates glutamate recycling and is important for maintaining GLU neurotransmission at fast firing frequencies. Unlike VGLUT2 cKO mice, GLS1 cHet mice (with a conditional heterozygous deletion of GLS1 in DA neurons) showed no alteration in DA content, DA release or DA neuron number. This allowed us to focus on the contribution of high frequency DA neuron GLU cotransmission, specifically during burst firing. We have recently shown that stimulation of DA neurons at burst firing frequencies can drive cholinergic interneurons (ChIs) in the NAc shell to burst fire, and that after a single dose of amphetamine, these DA neuron excitatory connections undergo dramatic drug-induced plasticity

(Chuhma et al., Neuron, 2014). We have now found, in DAT- IREScre::Ai32 mice and recording in CHs, that photostimulation of DA neuron terminals elicits GLU cotransmission that shows more rapid frequency dependent attenuation in GLS1 cHet (triple mutant) mice. Consistent with the overall lack of impact on DA transmission, GLS1 cHet mice showed no motor impairments measured on the rotarod, nor an altered dose-response curve for amphetamine-induced locomotion. Strikingly, GLS1 cHet mice did not show sensitization to repeated amphetamine. Thus, DA neuron GLU transmission at burst firing frequencies appears to underlie the development of drug-induced behaviors.

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779.16/141. ***Phosphorylation by PKC of the GluA1 subunit of the AMPA receptor in the nucleus accumbens is required for the expression of amphetamine sensitization***

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Two experiments assessed the contribution of PKC phosphorylation in the nucleus accumbens (NAcc) to the expression of locomotor sensitization by amphetamine. PKC constitutes a family of serine-threonine kinases that can be classified into three main isozyme subgroups with numerous substrates in the central nervous system. Some of these substrates especially in the NAcc could play a role in the expression of sensitization by amphetamine. In the first experiment, rats in different groups were exposed to injections of amphetamine (1.5 mg/kg, IP; injection every other day) and three weeks later, challenged with saline or amphetamine (1.0 mg/kg, IP). NAcc tissues were harvested 30 minutes later and PKC activity determined with a PepTag non-radioactive PKC assay using PLSRTLVAAK as peptide substrate or pNeurogranin(S36) as a postsynaptic endogenous substrate. In both cases, amphetamine exposure did not affect basal PKC activity but did enhance activity levels observed following the amphetamine challenge, suggesting a role for this kinase in the expression of locomotor sensitization by amphetamine. To further characterize this role, a second experiment assessed the need for phosphorylation by PKC of the S816-S818 residues of the GluA1 subunit of AMPA receptors in the NAcc. Phosphorylation of these residues is known to facilitate GluA1 insertion into the plasma membrane which in turn can enhance glutamatergic transmission and amphetamine-induced locomotion. Rats in different groups were exposed to saline and amphetamine as above. One week later, they were infected in the NAcc with lentivirus bearing GFP or the serine-alanine mutant GluA1(S816A-S818A) and two weeks after that tested for their locomotor response to NAcc amphetamine (2.5µg/side). Preventing GluA1 phosphorylation with the serine-alanine mutant blocked expression of the sensitized locomotor response to NAcc amphetamine normally observed in amphetamine exposed rats. Together, these results support the need for NAcc PKC in the expression of behavioral sensitization by amphetamine and highlight the importance of NAcc AMPA receptor regulation by PKC in generation of the sensitized response.

**779.17/142. *Exposure to amphetamine enhances AMPA receptor phosphorylation by CaMKII without increasing cell surface expression***

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While exposure to either cocaine or amphetamine leads to various manifestations of sensitization, only exposure to cocaine has been found to produce long-lasting increases in basal cell surface and synaptic AMPA receptor (AMPA) levels in rat forebrain. This led us to further characterize changes in AMPAR expression in the nucleus accumbens (NAcc) long after amphetamine exposure. In first series of experiments using the BS3 cross-linking approach, no difference between saline and amphetamine exposed rats was found in the cell surface expression of either GluA1 or GluA2 AMPAR subunits. This finding was supported in a second experiment showing no difference between these two groups in the amplitude of AMPAR-mediated mEPSCs recorded in NAcc slices. This finding was again confirmed in a third experiment showing no effect of amphetamine exposure in GluA1 and GluA2 levels observed either in whole cell lysate or in the PSD of NAcc neurons subjected to subcellular fractionation and Western blot assays. On the other hand and extending previous findings obtained in whole cell lysates, we found in a fourth experiment conducted in PSD fractions of NAcc shell tissues that exposure to amphetamine produces long-lasting increase in pGluA1(S831), a CaMKII residue. This increase in GluA1 phosphorylation was accompanied by a significant and long-lasting increase in levels of CaMKII $\alpha$ , but not CaMKII $\beta$ , in PSD fractions of the NAcc shell. This enrichment of CaMKII $\alpha$  in the PSD may provide one mechanism for the enduring increase in pGluA1(S831) which in turn can contribute to the enhanced behavioral responding to amphetamine and NAcc AMPA observed in sensitized rats. Finally, in an effort to delineate a mechanism underlying the increase in PSD CaMKII $\alpha$ , a fifth experiment using immunoprecipitation assessed the effect of amphetamine exposure on changes in protein coupling in the PSD. Interestingly, previous exposure to amphetamine was found to modestly increase the coupling of CaMKII $\alpha$  to PSD-95 but to significantly decrease CaMKII $\alpha$ /NR2b and increase NR2b/PSD95 coupling. Overall, these results reveal significant changes in protein interactions in the PSD of NAcc medium spiny neurons that are well positioned to influence AMPAR mediated signaling underlying the expression of behavioral sensitization by amphetamine. Some of the changes observed in protein coupling in the PSD are surprising given the need for increased CaMKII $\alpha$ /NR2b coupling in the expression of LTP. The decrease in CaMKII $\alpha$ /NR2b coupling observed in the present experiments likely characterizes a basal interactive state between these two proteins in periods removed from amphetamine challenge.

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**779.20/145. *Antisense-mediated downregulation of xCT reduces basal glutamate in the NA and alters post-synaptic AMPA receptor subunit expression***

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Ceftriaxone is a beta-lactam antibiotic which increases the expression and function of the glutamate transporter GLT-1 and of system xC-, which exchanges extracellular cysteine for intracellular glutamate. Basal glutamate levels in the NA (NA) are largely controlled by system xC-, and a decrease in its activity is contributing cause of the altered glutamate homeostasis observed in this brain region following cocaine self-administration in rats. The catalytic subunit of xC- is xCT, and we have demonstrated that expression of xCT and GLT-1 is decreased in the NA core following cocaine self-administration. We have also shown that ceftriaxone attenuates cue- and cocaine-primed reinstatement while restoring levels of both xCT and GLT-1 in the NA core. At this time it is not known if alterations in both transport systems mediate the altered synaptic plasticity in the NA after cocaine self-administration. Here we used a morpholino antisense strategy to decrease the expression of xCT protein and examined basal levels of glutamate and GluA1 and GluA2 expression. We found that xCT antisense infusion into the NA significantly decreased basal glutamate. We also found an increase in NA GluA1 expression in cocaine self-administering rats and no change in GluA2 expression. In ceftriaxone-treated rats infused with xCT antisense in the NA, this increase in GluA1 was potentiated. These data support the importance of xCT expression in maintaining basal glutamate in the nucleus accumbens and point to basal glutamate levels as key mediator of post-synaptic AMPA receptor alterations.

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780.01/147. ***Predicting treatment outcome in prescription opiate dependence using functional near infrared spectroscopy (fnir)***

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**Aims:** Growing evidence for a neuroadaptive model underlying long-term vulnerability to relapse in opiate dependence is largely based on animal research; little is known about its predictive capability concerning risk of relapse in clinical populations. The period directly following opiate withdrawal is marked by heightened CNS response to drug-related stimuli, diminished pleasure from natural rewards, increased stress response and irritability. The current study utilizes a cue reactivity paradigm coupled with fNIR to test the hypothesis that recently withdrawn prescription opiate dependent patients (RWP) in a residential setting would show reduced response to natural rewards and heightened response to drug-related stimuli. To date, relapse information has been gathered from a subset of patients, allowing us to examine the predictive value of this data. **Methods:** RWP (n=11) and healthy controls (HC; n=8) were evaluated using fNIR as they viewed hedonically positive (food), socially-relevant positive, drug related, or neutral stimuli. RWPs data was collected in a residential treatment facility, 10-14 days after withdrawal. T-tests and Pearson's correlations were used to compare fNIR response across groups. **Results:** RWPs showed reduced bilateral response to natural reward stimuli in the dorsolateral PFC (DLPFC) relative to HC (p<.05). Outcome data were available from a subgroup of RWP (n=8) that had either relapsed (n=4) or remained abstinent (n=4) for a period of three months following discharge from residential treatment. Relapse was associated with increased activation in the right DLPFC (p<.05) in response to food and drug stimuli, and decreased activation (p<.01) in bilateral DLPFC and ventromedial

PFC in response to socially rewarding stimuli. Responses to drug stimuli and socially rewarding were negatively correlated ( $r=-.727$ ;  $p=.041$ ) Conclusion: Consistent with previous studies, these data suggest RWPs display differential CNS responses to natural reward cues relative to HCs. Importantly, these preliminary data suggest that CNS responses in the PFC may serve as a biomarker to predict treatment outcome; this has the potential to be a powerful tool for clinicians (e.g., as an objective measure of vulnerability to relapse to guide treatment planning). These data demonstrate the feasibility of using fNIR, a relatively low-cost neuroimaging tool, in translational care.

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**780.02/148. *Functional magnetic resonance imaging measures of network connectivity related to incorrect responses predict completion of substance abuse treatment***

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US nationwide estimates indicate at least two-thirds of prisoners have a history of substance abuse or dependence. Tailoring substance abuse treatment to specific needs of incarcerated individuals could improve effectiveness of treating substance dependence and preventing drug abuse relapse. The purpose of the present study was to test the hypothesis that pre-treatment functional connectivity measures of error processing would predict which individuals would or would not complete a 12-week cognitive behavioral substance abuse treatment program. Adult incarcerated participants (N = 139; Females = 89) who volunteered for substance abuse treatment performed a response inhibition (Go/NoGo) functional magnetic resonance imaging (fMRI) task. Independent component analysis (ICA) was performed to identify networks related to false alarms elicited during the Go/NoGo task. Functional network connectivity measures of ICs related to false alarms were used to classify individuals who completed (N = 107; Females = 75) and discontinued (N = 32; Females = 14) treatment. A support-vector machine (SVM) classifier with a double input symmetric relevance feature selection step was used to identify two functional network connections that predicted treatment completion and discontinuation. For cross-validation, a nested 5-fold SVM with a radial basis function kernel was used to produce an overall accuracy of 81% while also identifying 81% of individuals who completed and 78% who prematurely discontinued treatment. Increased connectivity between the rostral anterior cingulate cortex (RACC) and striatal regions was measured in the completed group compared to the discontinued group. Increased connectivity between the caudal anterior cingulate cortex (CACC) and temporal regions (including insula) was measured in the discontinued group compared to the discontinued group. These findings support previous event-related potential (ERP) effects highlighting deficiencies in error-monitoring and post-error response adjustments in individuals who prematurely discontinue treatment. Cross-modal similarities suggest future treatments could be refined by targeting error-monitoring and post-error response adjustment in at-risk individuals, which could lead to more favorable long-term outcomes.

**780.09/J7. *The effects of N-Acetylcysteine on corticostriatal resting-state functional connectivity mediate nicotine withdrawal symptoms and may help prevent relapse***

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**BACKGROUND:** Chronic exposure to drugs of abuse disrupts corticostriatal glutamate transmission, which in turn mediates drug seeking. In animal models, N-acetylcysteine normalizes dysregulated frontostriatal glutamatergic neurotransmission and prevents reinstated drug seeking; however, the effects of N-Acetylcysteine on human corticostriatal circuitry function and preventing smoking relapse is unknown. **METHODS:** The present study examined the effects of N-Acetylcysteine on corticostriatal resting-state functional connectivity (rsFC), nicotine withdrawal symptoms and maintaining abstinence. Healthy adult smokers (N=16; mean (SD) age 36.5±11.9; cigs/day 15.8±6.1; yrs/smoking 15.7±8.9) were randomized to a double-blind course of 2400 mg N-Acetylcysteine (1200 mg b.i.d.) or placebo over the course of 3 ½ days of monetary-incentivized smoking abstinence. On each abstinent day, measures of mood and craving were collected digitally, and participants attended a lab visit in order to assess smoking (i.e., expired-air carbon monoxide [CO]). On day 4, participants underwent fMRI scanning. A demographically-matched nonsmoker control-group (N=16) was scanned once for comparison. **RESULTS:** As compared to placebo (n=8), smokers in the N-Acetylcysteine group (n=8) maintained abstinence, reported less craving and higher positive affect (PA) (all p's <.01), and concomitantly exhibited stronger frontostriatal and weaker visuoatriatal rsFC [p<.05; FWE]). Frontostriatal rsFC was negatively correlated with CO and fully mediated the relationship between craving and PA. Finally, no differences in rsFC or affect were found between the N-Acetylcysteine group and nonsmokers. **CONCLUSIONS:** Taken together, these findings suggest that N-Acetylcysteine may normalize dysregulated corticostriatal connectivity, help to restructure reward processing, and reduce vulnerability to relapse after quitting smoking.

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**780.12/J10. *Hormone dependent efficacy of taurine as treatment for cocaine drug use: study of reward***

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Cocaine is a commonly abused psychostimulant that causes alterations to the mesocorticolimbic circuitry and addiction-related behaviors. Females have been shown to be more vulnerable to the effects of cocaine than males. Taurine is an essential amino acid that displays several

neuropsychopharmacological roles such as neuromodulator, neurotrophic, and osmomodulatory roles. Previous data from our laboratory shows that males and females form preference to cocaine but pre-treatment of taurine attenuates cocaine preference to non-significant levels. The objective of the present study is to determine if exposure to taurine will reduce cocaine preference in gonadectomized (GDX) male and female subjects and elucidate whether gonadal hormones affect taurine's efficacy and potential for cocaine treatment. Males and females were run in two cohorts (n = 36; n=9/treatment group). The cohorts were divided into four groups. The first group are injected with taurine pretreatment and taurine+cocaine coadministration treatment (pre-tau/coc+tau), the second group is exposed to taurine pretreatment and cocaine+saline coadministration treatment (pre-tau/coc+sal), the third group also received the taurine pretreatment and taurine+saline coadministration treatment (pre-tau/tau+sal), and the fourth group is saline-pretreatment and cocaine+saline coadministration treatment (pre-sal/coc+sal). The rats are pretreated with taurine (100mg/kg) or saline (intraperitoneal) for two weeks before undergoing ten-day conditioned place preference (CPP) behavioral paradigm where a context will be paired with a drug stimulus (taurine or cocaine). GDX males acquired cocaine preference, and taurine inhibited cocaine reward under all conditions. GDX did not acquire a preference to taurine. Although GDX-females do not form a cocaine-preference, interestingly, GDX-females did acquire preference to the taurine-paired chamber (p<0.0064). These results suggest taurine is potentially a good candidate for cocaine addiction but further research will need to elucidate how hormones modulates taurine's efficacy since GDX-females seem to be sensitive to the effects of taurine. Cocaine-induced behaviors can persist for years even after abstinence and the best form of treatment is still being elucidated.

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780.13/J11. ***Putative dopamine agonist KB220Z enhances resting state brain reward circuit functional connectivity***

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Dopaminergic reward dysfunction in addictive behaviors is well supported in the literature. Several lines of evidence support the notion that alterations in synchronous neural activity between brain regions subserving reward and various cognitive functions, may significantly contribute to substance-related disorders. A electronic rat atlas was used to evaluate resting state functional connectivity in brain reward circuitry. This study presents the first strong evidence showing that a putative dopamine agonist nutraceutical (KB220Z) significantly activates, above placebo, seed regions of interest including the left nucleus accumbens, cingulate gyrus, anterior thalamic nuclei, hippocampus, pre-limbic and infra-limbic loci. This response induced by KB220Z demonstrates significant functional connectivity, increased brain

volume recruitment and enhanced dopaminergic functionality across the brain reward circuitry. This robust yet selective response implies clinical relevance.

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780.15/J13. ***Metabolism of L-tetrahydropalmatine (L-THP) in rats and In vitro effects of its major metabolites on dopamine receptors***

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L-THP is currently in clinical trial for treatment of cocaine addiction in the US. Here we reported its metabolism in rats and the in vitro pharmacology of its major metabolites. Following i.p. injection, L-THP was immediately absorbed into the blood and appeared in the brain. Metabolism of L-THP occurred rapidly with L-corydalmine (L-CD) and L-corypalmine (L-CP) as the two major metabolites, which peaked in the plasma at 15 min after i.p. injection. Following 30 mg/kg i.p. injection of L-THP, the plasma and brain levels were in the order of L-THP > L-CD > L-CP at all time points examined (from 15 min to 24 h). After 10 mg/kg i.p. injection, the plasma and brain levels were in the same order at two time points examined (30 min and 1 h). L-THP appeared to have first-order metabolism with a T<sub>1/2</sub> of about 2 h. The receptor binding and functional properties of L-CD, L-CP and L-THP on dopamine receptors were characterized using cell lines stably expressing each of the five human dopamine receptors (DR). [<sup>3</sup>H]SCH23390 binding and stimulation of adenylyl cyclase were used to examine the effects on D1R or D5R, and [<sup>3</sup>H]N-methyl-spiperone binding and [<sup>35</sup>S]GTPγS binding assays were for D2R, D3R or D4R. L-CD, L-CP and L-THP showed similar affinity for D1R (K<sub>i</sub>, 107-199 nM) and for D5R (K<sub>i</sub>, 242-313 nM). The K<sub>i</sub> values of L-CP to D2R and D3R were 42.4 and 262 nM, respectively, and of L-CD to D2R was 533 nM. The following interactions had low affinity (K<sub>i</sub> >1 μM): L-THP with D2R and D3R; L-CD with D3R; L-THP, L-CD and L-CP with D4R. For D1R and D5R, compared with dopamine, both L-CD and L-CP were partial agonists, with lower efficacies for D5R. For D2R and D3R, neither activated the receptors and exhibited antagonist activities. At the D2 receptor, L-CP and L-CD at 1 μM shifted the dose-response curve of DA to the right and increased the EC<sub>50</sub> value of DA by 15- and 5.8- fold, respectively. L-CP also reduced the E<sub>max</sub> value of DA significantly. At the D3 receptor, L-CP and L-CD at 1 μM shifted the dose-response curve of DA to the right and increased the EC<sub>50</sub> value of DA by 7.9- and 6.2-fold, respectively without changing the E<sub>max</sub> value of DA. Thus, both L-CD and L-CP are D1R and D5R partial agonists and D2R and D3R antagonists, with lower potencies at D2R and D3R. The effects of L-THP on DR-mediated signaling are currently under investigation.

780.16/J14. ***Dopamine D2 receptors as peripheral biomarkers for brain dopamine levels and as targets for modulating brain dopamine***

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Dopamine (DA) D2 autoreceptor (D2R) expression in the midbrain and striatum is a well-known biomarker for brain DA levels. Markers of D2R expression are not only detectable in the brain, but are also expressed in peripheral tissues, including blood, where DA appears to play a pivotal role in mediating communication between the nervous and immune systems. The aim of this study was to evaluate the expression of D2Rs on WBCs following exposure to DA and D2R agonists/antagonists, and to correlate expression with DA release in the nucleus accumbens (NAc) of the striatum, as well as locomotor and self-administration behavior. Using fluorescent flow cytometry and immunohistochemistry, D2Rs were expressed in WBCs in mice, rats and humans. However, their expression in rats was mostly limited to activated monocytes, which was the focus of this study. D2R expression in monocytes decreased at 1 hrs by 50% following administration of the centrally-acting D2R antagonist eticlopride (1.0 mg/kg IV) and increased at 1 hrs by 75% following administration of the centrally-acting D2R agonist quinpirole (0.1 mg/kg IV). However, D2R expression in the NAc exhibited opposite effects at 2 hrs to those in the blood. Concomitantly, using fast-scan cyclic voltammetry (FSCV), phasic DA release in the NAc was markedly enhanced 320% by eticlopride and reduced 76% by quinpirole with an onset in minutes and duration of 2 hrs. Surprisingly, intravenous administration of DA (0.1-3.0 mg/kg) enhanced DA release 2000% in the NAc with a lag of 2 min. This marked increase in DA release was not due to the blood pressure enhancing effects of DA via alpha adrenergic receptors. Intravenous DA resulted in complex behavioral effects depending upon dose, which included freezing at higher doses and activation at lower doses. Preliminary immunohistochemical studies have revealed expression of D2Rs in brain microglia after IV DA injection. These findings suggest that DAergic drugs not only yield rapid changes in brain D2 receptor expression, but elicit changes in peripheral D2 receptor expression that appear to be inversely correlated. In addition, activation of peripheral D2Rs may be a potential therapeutic target to raise brain DA levels. These results have significant clinical potential as changes in peripheral D2 receptor expression could be monitored as biomarkers of brain DA and used for the treatment of addiction as well as other diseases involving DA including Parkinson's disease.

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780.18/J16. ***Reinforcing and neurochemical effects differentiate modafinil from methylphenidate in their interactions with cocaine***

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Modafinil (MOD) and methylphenidate are FDA-approved as wakefulness-promoting agent, or for attention deficit disorder, respectively. They share with cocaine inhibition of dopamine (DA) reuptake by blockade of the DA-transporter, and have been tested in clinical studies as medications to treat cocaine-dependence. Non-medical use of MOD and methylphenidate as “smart drugs,” in order to increase cognitive performance, especially by students, has been described. This population is also at risk for abuse of illicit stimulants, like cocaine. Interactions of MOD and cocaine have not yet been fully described, and information about the potential adverse effects of MOD in combination with an abused psychostimulant like cocaine is of public health importance. In the present study MOD (0.1-10 mg/kg iv) failed to maintain self-administration behavior in rats, whereas methylphenidate self-administration was maintained at doses equal to those for cocaine self-administration (0.1-1 mg/kg iv). However, pretreatments with MOD (10-32 mg/kg ip) or methylphenidate (1-10 mg/kg ip) potentiated cocaine self-administration, shifting the dose-effect curves to the left. Cocaine, at self-administered doses also produced dose-related stimulation of DA in the nucleus accumbens shell, a brain area involved in the reinforcing effects of drugs. Methylphenidate enhanced this stimulation whereas MOD was without significant effects. In summary, MOD has a unique stimulant profile compared to cocaine and methylphenidate. The results suggest MOD may enhance cocaine-induced reinforcing effects. However, there are no clinical reports of abuse of modafinil alone or in combination with psychostimulants; thus, it remains to be seen if the recreational use of “smart drugs” such as MOD will lead to the abuse of illicit substances. Recent clinical studies report positive therapeutic outcomes of MOD treatment in cocaine addicted subjects. The results herein support that at the very least MOD may decrease cocaine consumption, if not entirely achieve abstinence. In the absence of any approved medication for this patient population, it seems that a medication that appears to have no addictive liability on its own, but could reduce cocaine use would be of interest.

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781.08/J24. ***Alternating access to a highly palatable diet and amphetamine sensitivity***

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Eating disorders and certain forms of obesity are associated with mesolimbic dopaminergic reward dysfunction. Overeating of diets rich in sugar and fat alter dopamine neurotransmission in the nucleus accumbens, likely contributing to the persistence of excessive intake. Amphetamine-like drugs, which act in the mesolimbic pathway to enhance the release of dopamine, also confer long-lasting neuroadaptations. However, it is not yet known if a history of disordered eating can alter sensitivity to these drugs. In this study, we investigated the effects of palatable food diet alternation on sensitivity to amphetamine challenges and amphetamine self-administration. Ad libitum diet alternation occurred for weeks prior to any testing. One group was provided chow diet days week, and second group was provided chow 5 days a week followed by 2 days of access to a highly palatable, chocolate flavored, high-sucrose diet. While continuing diet alternation, we measured locomotor activity following an

amphetamine challenge to test sensitivity during the 2 days of access to the palatable diet as well as when animals were withdrawn from palatable food. Following this within-subject amphetamine administration schedule, animals were allowed to self-administer amphetamine in water in the home cage. Rats withdrawn from intermittent access to palatable food exhibited pronounced hypophagia of the otherwise acceptable standard diet and overeating of palatable food upon renewed access. Further analysis is being conducted to understand how history of overconsumption of palatable food might sensitize the mesolimbic pathway to the stimulatory effects of amphetamine-like drugs, and whether this may be augmented during periods of palatable food withdrawal.

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781.15/J31. ***Binge-like eating as an addiction-like disorder: Novel findings from an operant model in rats***

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Binge eating disorder is characterized by excessive consumption of highly palatable food within short periods of time accompanied by loss of control over eating. Extensive evidence provides support for the consideration of binge eating disorder as an addiction-like disorder. In this study, we wanted to determine whether rats undergoing an operant binge-like eating procedure could develop maladaptive forms of conditioned feeding behaviors. We also wanted to determine whether the binge eating procedure could alter dopamine D2 receptor (D2R) levels in key brain regions implicated in drug and natural reward processing. For this purpose, we trained male rats to self-administer either sugary, highly palatable diet ("Palatable" rats) or chow diet ("Chow" rats) for 24 hour day. After escalation and stabilization of palatable food intake, we tested Chow and Palatable rats in (a) a conditioned place preference test, (b) a second-order schedule of reinforcement and (c) a cue-induced suppression of feeding test. In the conditioned place preference task, Palatable rats spent significantly more time in the compartment that was previously paired with the palatable food, compared to Chow controls. Furthermore, in the second-order schedule of reinforcement task, Palatable rats exhibited active lever responding 4- to 6-fold higher than Chow control rats. In addition, in the cue-induced suppression of feeding test, although Chow control subjects reduced responding by 32% in the presence of the conditioned punishment, Palatable rats persevered in responding despite the aversive cue. Finally, following exposure to the palatable diet, binge-eating rats showed reduced D2R levels in prefronto-cortical regions of the brain. These results further characterize this animal model of binge-like eating and provide additional evidence for the addictive properties of highly palatable food.

782.02/J33. **Exploring a putative protein: Protein interaction between the serotonin (5-HT) 5-HT2A receptor (5-HT2AR) and 5-HT2CR**

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The serotonin (5-HT) 5-HT2A receptor (5-HT2AR) and 5-HT2CR play important roles in behavior and physiology. We have recently demonstrated that knockdown of 5-HT2CR following microinjection of 5-HT2CR shRNA into the rat medial prefrontal cortex (mPFC) evokes a behavioral phenotype characterized by increased motor impulsivity and elevated reactivity to cues associated with cocaine self-administration. The 5-HT2CR knockdown in mPFC also resulted in upregulation of 5-HT2AR protein in the mPFC and a leftward shift in potency of systemic M100907 to suppress motor impulsivity, suggesting functional disruption of local 5-HT2AR:5-HT2CR balance. Furthermore, co-immunoprecipitation studies suggested that a protein:protein interaction exists between 5-HT2AR and 5-HT2CR in mPFC. In the present study, we employed immunohistochemistry, proximity ligation assay (PLA), and luciferase complementation assay (LCA) technologies in either live cells and/or rat brain, as well as the in silico direct coupling analysis (DCA), to test the hypothesis that a protein:protein interaction occurs between the 5-HT2AR and 5-HT2CR. In immunohistochemical analyses, we found that 5-HT2AR and 5-HT2CR protein co-localized within the same cells in rat mPFC. In the PLA (Duolink), we found that native, unmodified proteins are in close proximity (<45 nm) in mPFC. In the DCA (which examines the co-evolution of residues in over 10<sup>5</sup> species), we identified candidate pairs of amino acid residues that are predicted to be in direct functional contact, most notably between the extracellular N-terminus domains of the two proteins. The LCA is being employed to test the hypothesis that the N-terminal domains are the primary points of interaction between the two receptors. In the LCA, two complementary luciferase N- (NLuc) and C-terminus (CLuc) fragments, which have no activity on their own, are fused to the two receptor proteins of interest, respectively. In the presence of the substrate D-luciferin, association of the two proteins brings the inactive fragments into close proximity to reconstitute the enzyme activity. We are co-expressing 5-HT2AR-NLuc (or CLuc) and 5-HT2CR-CLuc (or NLuc), expressed on the N- or C-terminus in mammalian cells to demonstrate the formation of a protein:protein interaction between the 5-HT2AR and 5-HT2CR in live cells. We will treat the cells with a peptide which is predicted by the DCA to disrupt the interaction and impact cellular signaling. To date, our findings suggest that 5-HT2AR:5-HT2CR protein interaction may provide a new neurobiological mechanism underlying behavior and a possible target for novel pharmacotherapeutics, such as heterobivalent ligands.

782.03/J34. ***Serotonin (5-HT) 5-HT2A receptors in the medial prefrontal cortex regulate cue reactivity following prolonged forced abstinence from cocaine self-administration***

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An intensification of craving in humans and reactivity to drug-associated cues in rodents occurs during abstinence while elevation of cue reactivity (“incubation”) is observed in rats exposed to prolonged periods of forced abstinence from cocaine self-administration. Incubation in rodents has been linked to time-dependent neuronal plasticity in the medial prefrontal cortex (mPFC). The mPFC expresses the serotonin (5-HT) 5-HT<sub>2A</sub> receptor (5-HT<sub>2AR</sub>) and blockade of the mPFC 5-HT<sub>2AR</sub> suppresses cue reactivity. We have demonstrated that membrane expression of the 5-HT<sub>2AR</sub> protein in the mPFC predicts levels of cue reactivity [lever presses reinforced by the discrete cue complex (e.g., stimulus light, pump tone)] following cocaine self-administration in rats. With regard to incubation, we found that expression of the 5-HT<sub>2AR</sub> is elevated in membrane extracts of the mPFC on Day 30 vs. Day 1 of forced abstinence from cocaine self-administration. This observation prompted us to test the hypothesis that rats will be more sensitive to the effects of a selective 5-HT<sub>2AR</sub> antagonist (M100907) to suppress cue reactivity on Day 30 vs. Day 1 of forced abstinence. Rats were trained to self-administer cocaine for 14 days (0.75 mg/kg/inf, FR5) and the effects of M100907 (0.03-0.3 mg/kg, i.p.) on cue reactivity were measured on Day 1 and Day 30 of forced abstinence. Cue reactivity was significantly elevated on Day 30 vs. Day 1 of forced abstinence. Pharmacological analysis revealed that all doses of M100907 (0.03-0.3 mg/kg) significantly ( $p < 0.05$ ) suppressed cue reactivity during prolonged (Day 30), but not early abstinence (Day 1). These data suggest that the elevated expression of membrane 5-HT<sub>2AR</sub> protein in the mPFC on Day 30 may contribute mechanistically to the time-dependent incubation of cue reactivity following forced abstinence. Mechanistically linking 5-HT<sub>2AR</sub> regulatory mechanisms to time-dependent incubation phenomena will have direct clinical implications in developing 5-HT<sub>2AR</sub>-targeted therapeutics to minimize vulnerability to relapse in cocaine use disorder.

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782.04/J35. ***Serotonin (5-HT) 5-HT2A receptor (5-HT2AR):5-HT2CR protein interaction as a therapeutic target: Novel 5-HT2AR antagonist/5-HT2CR agonist heterobivalent ligands as neuroprobes***

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A feature of multiple neuropsychiatric disorders is motor impulsivity. Recent studies have implicated 5-HT system in medial prefrontal cortex (mPFC) in mediating individual differences in motor impulsivity, notably the 5-HT<sub>2AR</sub> and 5-HT<sub>2CR</sub>. We investigated the hypothesis that differences in the ratio of 5-

HT2AR:5-HT2CR in mPFC would predict the individual level of motor impulsivity. Native protein levels of the 5-HT2AR and the 5-HT2CR predicted the intensity of motor impulsivity and the 5-HT2AR:5-HT2CR ratio in mPFC tracked with levels of premature responses in individual outbred rats. The possibility that the 5-HT2AR and 5-HT2CR act in concert to control motor impulsivity is supported by the observation that high motor impulsivity associated with a diminished mPFC synaptosomal 5-HT2AR:5-HT2CR protein interaction. We infer that there is an interactive relationship between the mPFC 5-HT2AR and 5-HT2CR, and that 5-HT2AR:5-HT2CR imbalance may be a functionally-relevant mechanism underlying motor impulsivity. Based on these findings, we propose that correcting this imbalance and stabilizing the 5-HT2AR:5-HT2CR protein interaction utilizing a ligand with dual action as a selective 5-HT2AR antagonist (e.g., M100907)/5-HT2CR agonist (e.g., WAY163909) presents a promising and novel pharmacotherapeutic strategy for treatment of brain disorders in which motor impulsivity is implicated. We identified benign tether locations on M100907 and WAY163909 and used click chemistry to synthesize a series of M100907:WAY163909 heterobivalent ligands. We employed quantitative live cell assays to measure two types of signaling evoked by ligand activation,  $\text{Ca}^{2+}$  release and phosphorylation of ERK1/2. In the 5-HT2AR-CHO-K1 cell line, the heterobivalent ligand inhibited 5-HT-induced  $\text{Ca}^{2+}$  release with IC50s ranging from 125-210 nM. In the 5-HT2CR-CHO-K1 cell line, the heterobivalent ligands demonstrated no intrinsic activity, however, in the dual expressing 5-HT2A+2CR-CHO-K1 cell line, the heterobivalent ligands inhibited 5-HT-induced  $\text{Ca}^{2+}$  release with IC50s between 438-725 nM. Interestingly, the heterobivalent ligands appear to act as partial agonists given their ability to phosphorylate ERK1/2 in the 5-HT2A+2CR-CHO-K1 cell line, demonstrating a novel pharmacological profile in vitro. Extensive behavioral analyses are currently underway to evaluate our novel heterobivalent molecules in vivo. These data provide the first profile of heterobivalent 5-HT2AR antagonist/5-HT2CR agonist ligands which will be useful as neuroprobes and potentially useful to develop treatment strategies for brain disorders in which impulsivity is a factor.

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782.05/J36. ***Dynamic regulation of synaptosomal serotonin (5-HT) 5-HT2C receptor (5-HT2CR) expression following acute cocaine administration***

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Activation of the 5-HT2CR in the medial prefrontal cortex (mPFC) regulates cocaine-seeking assessed in the cocaine self-administration/forced abstinence assay in rats. The functional impact of 5-HT2CR activation is mechanistically controlled by a complement of factors, including subcellular localization of the receptor. Previous studies indicate that the 5-HT2CR is poised to influence the synaptosomal milieu in the mPFC and interfaces in a functionally-coordinated manner with synaptic proteins to promote or inhibit receptor readiness and subsequent signaling capacity. We previously demonstrated that synaptosomal 5-HT2CR expression in the mPFC on Day 30 of forced abstinence is lower than on Day 1,

suggesting that regulation of the 5-HT<sub>2</sub>CR in mPFC may be a mediator of elevated cue-elicited cocaine-seeking in late abstinence from cocaine self-administration. Interestingly, the levels of 5-HT<sub>2</sub>CR expression in the synaptosomal compartment of the mPFC of naïve rats mirrors those assessed at Day 30; levels observed at Day 1 are significantly elevated relative to naïves. These data suggest that 5-HT<sub>2</sub>CR protein expression, and perhaps subcellular localization of the receptor, are responsive to the pharmacological environment consequent to recent self-administration of cocaine and/or acute withdrawal. In the present study, we tested the hypothesis that acute pretreatment with or withdrawal from non-contingent cocaine will increase synaptosomal 5-HT<sub>2</sub>CR expression in a time-dependent manner. Male Sprague-Dawley rats were injected with saline (0.9%; i.p.) or cocaine (15 mg/kg; i.p.) 15 min or 24 hrs prior to sacrifice and mPFC harvest. Crude synaptosomal protein was extracted from the mPFC and 5-HT<sub>2</sub>CR protein expression was measured using a novel medium-throughput 96-well plate immunoassay adapted for use with brain tissue in our laboratory. The plate immunoassay is highly reliable, reproducible and amenable to assaying multiple conditions in the same experiment. We found that synaptosomal 5-HT<sub>2</sub>CR expression in mPFC was elevated 15 min following cocaine vs. saline administration; analyses at 2 hrs of withdrawal are in progress. Ongoing experiments are exploring the association of the 5-HT<sub>2</sub>CR with synaptic protein partners (e.g. proteins vs.  $\beta$ -arrestins) which regulate its trafficking and function. Understanding the dynamic regulation of 5-HT<sub>2</sub>CR expression following acute cocaine administration is a critical first step toward exploring the effects of chronic cocaine-taking and withdrawal on 5-HT<sub>2</sub>CR expression, which will ultimately inform the development of effective pharmacotherapeutics to treat cocaine use disorder.

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782.06/J37. ***Individual differences in impulsive action in rats are governed by cortical N-methyl-D-aspartate receptor (NMDAR) tone***

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Impulsivity is a complex, multifaceted trait broadly defined as action without sufficient foresight; high inherent impulsivity may increase the likelihood that drug use escalates into dependence and relapse. Glutamate neurotransmission within the medial prefrontal cortex (mPFC) may critically regulate the cognitive and/or behavioral dimensions underlying impulsivity. The N-methyl-D-aspartate receptor (NMDAR) is a member of the ionotropic glutamate receptor family and is composed of multiple subunits including GluN1 and GluN2A-D. Noncompetitive antagonism of the NMDAR and selective antagonism of the GluN2B subunit enhances impulsivity in animal models. Here, we tested the hypothesis that differences in NMDAR subunit composition and signaling deficits within the mPFC mediate high inherent impulsivity, and that pharmacological potentiation of NMDAR signaling attenuates high inherent impulsivity. Outbred male Sprague Dawley rats were identified as high (HI) or low (LI) impulsive using the one-choice serial reaction time (1-CSRT) task. In this task, nose-pokes after presentation of a visual stimulus resulted in food pellet delivery. Nose-pokes before presentation of the visual stimulus (i.e., premature responses) indexed impulsive action. quartile split based on the number of premature

responses was used to identify HI or LI rats. Following phenotypic identification, mPFC synaptosomal protein was extracted from a cohort of HI and LI rats to assess NMDAR composition via immunoblotting.

separate cohort of HI and LI rats were trained to criterion on the 1-CSRT task, and on test days, pretreated with vehicle (saline, 1 ml/kg; i.p.) or D-cycloserine (DCS; agonist at strychnine-insensitive glycine site of NMDAR; 1-50 mg/kg, i.p.). Performance on the 1-CSRT task was rapidly acquired and allowed stable identification of HI and LI rats; premature responses in HI rats remained significantly higher than LI rats across 70 training days ( $p < 0.001$ ). HI rats had lower mPFC GluN1 and GluN2A synaptosomal protein expression compared to LI rats ( $p < 0.05$ ). No difference in GluN2B levels was detected between HI and LI rats. Select doses of DCS decreased premature responses relative to saline administration in HI, but not LI, rats ( $p < 0.05$ ). Taken together, inherent impulsive action may be critically driven by dysregulation of mPFC GluN1/GluN2A signaling and selective potentiation of NMDAR function may rescue high inherent impulsive action. Increased understanding of the neurobiology underlying inherent differences in impulsivity may aid development of pharmacotherapies that target drug dependence, relapse, and other disorders characterized by impulsivity.

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**782.07/J38. *Activation of the corticoaccumbens circuit attenuates inherent impulsivity and binge intake of high fat food***

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Eating is essential for life, but repeated consumption of large amounts of food in a brief period (i.e., bingeing) can alter the reward value of food and food-related cues and fuel binge-eating cycles. Impulsivity, a predisposition toward rapid unplanned reactions to stimuli, is one of the multifaceted determinants underlying the etiology of dysregulated eating, its pathogenesis, and treatment outcomes. The medial prefrontal cortex (mPFC) is a major neural director of reward-driven behavior, impulse control and integration of internal states with environmental cues. Compromised signaling between the mPFC and nucleus accumbens (NAc) are thought to underlie the cognitive inability to withhold prepotent responses (impulsive action) and binge intake of high fat food. We propose that strategies to directly activate this circuit would indicate that the ventral infralimbic (IL)-nucleus accumbens shell (NAcSh) pathway plays a putative suppressive role (“Stop”) in impulsive action and binge eating. We employed a dual viral vector technology that allows for the targeted and isolated modulation of IL mPFC neurons that project to the NAcSh via a Cre-loxP system. A Cre-dependent viral vector based “double-floxed” inverted open reading frame (DIO) switch system expresses an engineered Gq-DREADD which only binds clozapine-N-oxide (CNO). In the presence of Cre, the loxP sites are excised and the transgene is inverted into the sense direction and expressed from the hSyn promoter. An AAV DIO construct that contains an inverted version of Gq DREADD (hM3D)-mCherry or mCherry alone was infused into the IL

mPFC. A canine adenovirus-2 (CAV)-Cre axonal retrograde viral vector was infused into the NAcSh of the same rat; stable transgene expression in IL mPFC occurred only at the site of DIO vector infusions thus restricting expression to cortical neurons that project to the NAcSh. Activation of the circuit with DIO-hM3D-mCherry AAV in the presence of CNO significantly suppressed impulsive action in the 1-choice serial reaction time task ( $p < 0.05$ ); no differences in task acquisition, accuracy, omissions or additional task parameters were observed. The DIO-hM3D-mCherry-AAV in the presence of CNO significantly decreased binge intake for high fat food ( $p < 0.05$ ). These data indicate that impulsive action and binge eating reciprocally interact at the level of an imbalance in homeostasis within the corticoaccumbens circuit. Through addressing a fundamental gap in our knowledge of how the neural aspects of impulsivity relate to binge eating, we hope to develop pharmacological strategies to minimize binge eating and enhance clinical practice for disorders of overeating.

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**782.09/J40. Embryonic methamphetamine exposure inhibits methamphetamine cue conditioning and reduces dopamine tissue levels in adult wild-type *C. elegans***

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Methamphetamine (MAP) addiction is a serious and costly disorder leading to thousands of deaths and costing billions of dollars annually. A disturbing outcome of MAP addiction is the uncontrolled embryonic exposure to this drug and the effects on brain development and behavioral consequences later in life. Although studies have begun to provide important information about some aspects of the neurobiology of MAP addiction, very little research has focused on these developmental issues and few good developmental models are available. *C. elegans* is an excellent model organism to study the neurobiological consequences of embryonic MAP exposure. It has a well studied and described neuroanatomical system, along with a short generation time for fast generation of data at a fraction of the cost of many other organisms. The objective of the current study was to determine the long-term behavioral and neurochemical effects of embryonic MAP exposure in *C. elegans*. Wild-type N2 worms were embryonically-exposed to 50  $\mu$ M MAP or vehicle. Using classical conditioning, embryonically-exposed worms were conditioned to MAP (17 and 50  $\mu$ M) as adults in the presence of either sodium ( $\text{Na}^+$ ) or chloride ( $\text{Cl}^-$ ) ions as conditioned stimuli (CS+/CS-). Following conditioning, a preference test was performed by placing worms in 6-well test plates spotted with the CS+ and CS- at opposite ends of each well. A food conditioning experiment was also performed to determine if embryonic MAP exposure affected food conditioning behavior. For the neurochemical experiments, adult worms that were embryonically-exposed to MAP were analyzed for dopamine (DA) content using high performance liquid chromatography. Pairing an ion with 17 and 500  $\mu$ M MAP significantly increased the preference for that ion (CS+) by  $181 \pm 15 \%$  and  $176 \pm 18 \%$  of controls, respectively, in worms that were not pre-exposed to MAP. However, worms embryonically-exposed to MAP did not exhibit significant drug cue conditioning. The inability of MAP-exposed worms to condition to MAP was not associated with deficits in food conditioning, as MAP-exposed worms exhibited significant cue preference associated with food.

Furthermore, embryonic MAP exposure reduced DA levels by 2 to 37% of baseline levels in adult *C. elegans*, which could be a key mechanism contributing to the long-term effects of embryonic MAP exposure. Overall, these data suggest that embryonic MAP exposure selectively reduces the reinforcing properties of MAP in adult *C. elegans*, which may be driven in part by concomitant decreases in DA levels. Supported by DA035468(EAE).

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**782.11/J42. *Differential effects of translin deletion on behavioral and biochemical responses to cocaine and amphetamine***

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To help define the role of the microRNA system in regulating neuronal signaling, we have studied the impact of translin deletion on the dopamine system. Translin and its partner protein, trax, form an RNase complex that mediates degradation of microRNAs (Asada et al., Cell Reports, 2014). Accordingly, we have used translin KO mice to assess the role of the microRNA system in dopamine signaling. We have found that the classic ability of cocaine to increase open field exploration is markedly inhibited in these mice. Furthermore, the ability of cocaine to increase nucleus accumbens dopamine levels as monitored by microdialysis is severely compromised. Accordingly, these findings suggest that elevated microRNA levels caused by translin deletion impair dopamine release from mesolimbic dopamine neurons. In contrast, the locomotor response to amphetamine is increased in these mice, while the ability of amphetamine to elevate dopamine levels in the nucleus accumbens is normal. These findings indicate that the enhanced locomotor response to amphetamine is due to post-synaptic supersensitivity to dopamine, which may be secondary to reduced dopamine tone. Current studies are aimed at assessing whether reinstatement of translin expression in VTA dopamine neurons is sufficient to rescue these phenotypes. In summary, these findings suggest that inhibition of the translin/trax complex provides a novel means of decreasing dopamine tone and may have therapeutic potential by promoting resilience in the face of chronic stress.

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**782.13/J44 *Physical and emotional stress alter voluntary morphine consumption ventral tegmental area gene expression***

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There is significant co-morbidity of mood disorders and drug dependence, but the mechanisms contributing to this co-morbidity are not well understood. Preclinical models of mood disorders typically employ chronic stress to elicit depressive-like behaviors. Here, we used physical chronic social defeat

stress (CSDS), as well as a modified version, emotional CSDS, to investigate changes in morphine reward and to identify potential molecular mediators of stress susceptibility and reward. We subjected male mice to standard 10-day physical or emotional CSDS and assessed social interaction (SI) on day 11 followed by voluntary morphine consumption using a two-bottle choice assay. Both physical and emotional CSDS decrease SI score on day 11, with physical stress eliciting more robust social avoidance than emotional stress. Physical and emotional CSDS also significantly increase morphine preference and there is a significant negative correlation between SI score and morphine preference. Given that CSDS induces long-lasting changes in SI, we examined whether changes in morphine reward persist. We observed a similar significant negative correlation between SI score and morphine consumption 14 days after the last defeat. Next, we determined whether morphine preference was also altered during emotional CSDS. Contrary to results following stress, mice undergoing emotional CSDS showed a trend for decreased morphine preference compared to controls. Finally, we wanted to determine whether individual differences in morphine preference could predict susceptibility to CSDS. We found that morphine preference (determined 14 days prior to stress) did not predict susceptibility to CSDS. Combined, these data suggest that CSDS differentially affects morphine reward, depending on when consumption is measured. Given the importance of the ventral tegmental area (VTA) in both CSDS and drug reward, we are currently investigating drug- and CSDS-induced changes in VTA gene expression as potential mediators of these behavioral effects. One promising candidate is serum- and glucocorticoid-regulated kinase 1 (SGK1) as we have found that chronic morphine and physical and emotional CSDS significantly increase SGK1 gene expression in the VTA. We also have preliminary data suggesting that SGK1.1, a brain-specific isoform of SGK1 known to influence neuronal excitability, exhibits a positive correlation with SI score. The decreased expression of SGK1.1 in susceptible mice could contribute to VTA activity changes that influence CSDS susceptibility. Current studies are investigating the role of SGK1, as well as other novel molecules, in drug- and stress-induced changes in the VTA.

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782.15/J46. ***Stress acutely promotes calcium-dependent glutamatergic synaptic plasticity in the VTA via differential actions of CRF and norepinephrine***

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Stressful life experiences are a well-known risk factor for the development of addiction. Addiction is driven, in part, by powerful and enduring memories of sensory cues experienced during drug intake. Addictive drugs are thought to hijack synaptic plasticity mechanisms in key brain circuits involved in reward learning, especially the mesolimbic dopaminergic system that originates in the ventral tegmental area (VTA). Glutamatergic inputs activating NMDA receptors (NMDARs) drive dopamine neuron burst firing. Therefore, long-term potentiation of (LTP) of NMDAR-mediated transmission in the VTA may contribute to the increased motivational valence of drug-associated cues. In this study, we tested the hypothesis that stress acutely enhances the induction of NMDAR LTP via rapid, short-term actions of corticotropin-releasing factor (CRF) and norepinephrine (NE), the two key mediators of acute stress

responses in the CNS. Mechanistically, LTP induction requires burst-evoked Ca<sup>2+</sup> signals amplified by preceding activation of the metabotropic glutamate receptor (mGluR)-inositol triphosphate (IP<sub>3</sub>) pathway. Using rat VTA slices, we first examined how CRF and NE regulate this Ca<sup>2+</sup> signal amplification process. We found that bath application of CRF, while having no effect by itself, significantly enhanced facilitation of burst-evoked Ca<sup>2+</sup> signals caused by direct photolytic application of IP<sub>3</sub> into the cytosol. In contrast, NE or the  $\alpha$ <sub>1</sub>-adrenergic receptor agonist phenylephrine (Phe), increased burst-evoked Ca<sup>2+</sup> signals by themselves, as  $\alpha$ <sub>1</sub>-adrenergic receptors are coupled to IP<sub>3</sub> generation. Furthermore, Phe-induced Ca<sup>2+</sup> signal facilitation was augmented by co-application of CRF. As a consequence, CRF and Phe differentially promoted NMDAR LTP induction, via facilitation of IP<sub>3</sub> effect and generation of IP<sub>3</sub>, respectively, in a cooperative manner. Finally, we found that single and transient exposure to social defeat stress before conditioning resulted in enhanced acquisition of cocaine-conditioned place preference. These results suggest that stress acutely promotes learning of the motivational valence of drug-associated cues via CRF/NE-induced enhancement of glutamatergic synaptic plasticity in the VTA.

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782.16/J47. ***Investigation of biochemical changes induced by chronic morphine and stress in the ventral tegmental area***

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Drug addiction and depression are two co-morbid diseases that have a significant health and financial burden on society. Increasing evidence suggests that dysfunction of the mesolimbic dopamine (DA) pathway could underlie this co-morbidity. We are investigating the biochemical mechanisms that drive changes in the morphology of ventral tegmental area (VTA) DA neurons induced by chronic morphine and chronic social defeat stress (CSDS). Our previous work demonstrated that exposure to chronic opiates decreases VTA DA neuron soma size in rodents and that this change is dependent on mammalian target of rapamycin complex 2 (TORC2) signaling. Moreover, decreased soma size and TORC2 signaling correlates to changes in opiate reward, highlighting the importance of understanding this process. Preliminary evidence from our lab suggests that mice that are susceptible to CSDS also have a significant decrease in VTA DA soma size. Given these similar alterations in VTA DA soma size, we are seeking to identify the molecular mechanisms. One promising candidate is the Rac1 pathway, which is a well-known mediator of actin cytoskeleton remodeling. Rac1 activity has also recently been linked with TORC2 signaling, as knockout of the TORC2 constituent protein Rictor decreased Rac1 signaling and hippocampal spine density (Huang et al. 2013). Given that chronic morphine decreases TORC2 signaling in the VTA, we are currently determining whether Rac1 activity is also decreased. We have performed western blot analysis on micro-dissected VTA tissue from mice exposed to chronic morphine or CSDS and find a significant decrease in the phosphorylation of cofilin, a protein involved in severing actin filaments, which is downstream of Rac1. We are now examining molecules upstream of cofilin to determine if exposure to chronic morphine or stress similarly alters activity of these proteins.

Additionally, in order to evaluate whether changes in VTA TORC2 signaling are sufficient to alter Rac1 activity, we are conducting western blot analysis of VTA tissue from mice with knockout or viral-mediated overexpression of Rictor. These studies will determine whether decreasing or increasing VTA TORC2 signaling decreases or increases Rac1 signaling, respectively. Thus, the ultimate aim of these studies is to establish the molecular mechanisms underlying VTA DA structural plasticity in the hopes of identifying potential novel targets for therapeutic intervention in drug addiction and depression.

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782.17/J48. ***Acute morphine administration induces a partly reversible long term depression of inhibitory synaptic transmission onto dopaminergic ventral tegmental area neurons***

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Dopaminergic neurons in the ventral tegmental area (VTA) play an essential role in mediating the acute effects of drugs of abuse. Furthermore, synaptic abnormalities in the VTA following consumption of drugs of abuse likely promote addiction. Our laboratory previously found that a single injection of morphine is sufficient to impair the induction of GABAergic synaptic plasticity onto dopaminergic VTA neurons 24 hours after injection. Here we report that a single morphine injection per se induces a long term depression (LTD) of GABAergic transmission onto dopaminergic VTA neurons. This morphine-induced LTD is present both presynaptically and postsynaptically as evidenced by a decrease in both amplitude and frequency of miniature inhibitory postsynaptic currents (mIPSCs). We then attempted to recover these abnormalities using CL-994, class I histone deacetylase (HDAC) inhibitor that we recently demonstrated could recover synaptic abnormalities in the VTA induced by an episode of early life stress. two hour incubation of rat midbrain slices in Cl-994 managed to recover mIPSC amplitudes to normal levels. These results suggest that the acute effects of morphine administration may in part be mediated by epigenetic mechanisms, namely histone deacetylation.

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784.02/K19. ***Serotonin receptor 2 agonists lorcaserin and CP-809101 block cue-induced reinstatement of sugar-seeking behavior in rats***

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main challenge for those intending to lose weight by dieting is to resist the temptation of preferred, highly caloric foods despite continued exposure to food and with cues associated with food. Manipulations of serotonin (5-hydroxytryptamine, 5-HT) signaling have been shown to affect a variety of motivated behaviors. In particular, the 5-HT<sub>2C</sub> receptor has been implicated as a therapeutic target for

weight loss, and lorcaserin (Lorquess; Belviq) has been approved for the treatment of obesity. However, although the anorectic effects of 5-HT<sub>2C</sub> receptor stimulation have been well-described, recent research has also shown that 5-HT<sub>2C</sub> agonists reduce reinstatement of nicotine-seeking in the presence of drug cues, which suggests that lorcaserin may also aid in weight loss due to inhibition of food-seeking in the presence of food-associated cues. In these experiments, we examined the impact of lorcaserin and the highly selective 5-HT<sub>2C</sub> agonist CP-809101 on 1) cue-induced reinstatement of sugar-seeking behavior, and 2) 2-hr food intake in rats on a 22-h deprivation schedule. Male Sprague-Dawley rats (N = 12/group) were food-deprived and trained to lever press for sugar pellets in the presence of a light/tone cue (for details, see Lin & Pratt, 2013). Once trained, rats underwent daily 20-min extinction sessions in which lever presses resulted in no programmed consequences. Upon extinguishing to 10% of their pre-extinction performance, rats received two separate reinstatement sessions following drug or saline injections (counterbalanced across rats and separated by 48 hr) in which lever pressing resulted in delivery of the light/tone cue. Prior to these reinstatement tests, rats received SC injections of saline or drug (Groups 1-3: lorcaserin at 0.1, 0.3, & 0.6 mg/kg; Group 4; CP-809101 at 1 mg/kg). Lorcaserin at 0.3 and 0.6 mg/kg (but not 0.1 mg/kg) significantly blocked cue-induced reinstatement of lever pressing to the sugar-associated cues, as did the 1 mg/kg of CP-809101 (e.g., reinstatement under vehicle: 88.4±13.4 lever presses; Lor 0.6 mg/kg: 43.2±6.5 lever presses; CP 1 mg/kg: 48.0±9.5 lever presses). Notably, these effects occurred at doses of the 5-HT<sub>2C</sub> receptor agonists that were subthreshold to the anorectic effects of the drugs, as none of the doses tested in the reinstatement paradigm affected 2-hr food intake in animals tested under a 22-hr food deprivation schedule (e.g. Veh: 21.3±0.9g, Lor 1 mg/kg: 21.2±1.3g; NS). These data suggest that the therapeutic efficacy of 5-HT<sub>2C</sub> receptor agonists may include inhibition of the appetitive aspects of food-directed motivation.

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784.06/K23. ***Intrahippocampal injection of a small molecule RGS4/19 inhibitor has antidepressant-like effects in a mouse model***

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Regulators of G-protein signaling (RGS) proteins are a large family of intracellular proteins that negatively regulate G-protein coupled receptor (GPCR) signaling to heterotrimeric G proteins. RGS proteins act as GTPase accelerating proteins (GAPs) and interact directly with active GTP-bound G $\alpha$  subunits. This action enhances the hydrolysis of GTP to GDP, thus inactivating the G $\alpha$  subunits and allowing reformation of the inactive G $\alpha$ / $\beta$ / $\gamma$ -protein heterotrimer. This allows RGS proteins to reduce both the duration and extent of GPCR signaling. We have demonstrated that mice expressing an RGS insensitive variant of G $\alpha$ i2 (RGSi G $\alpha$ i2; knock-in variant of G $\alpha$ i2 with a single point mutation in the switch region that prevents interaction with all RGS proteins) exhibit an antidepressant-like phenotype in several behavioral tests. This phenotype is caused by increased signaling downstream of 5-HT<sub>1A</sub> receptors (5-HT<sub>1A</sub>R). In addition, we have shown in cultured hippocampal neurons that RGS19 is an effective inhibitor of 5HT<sub>1A</sub> receptor signaling. Furthermore evidence suggests that RGS4 and RGS6 are

important modulators of antidepressant drug action. The present study sought to answer two questions: A) What is the location of the 5HT1ARs involved in the antidepressant-like phenotype in the RGSi Gai2 mice and B) What information can be gained about the specific RGS proteins that are responsible for negative regulation of these 5HT1ARs. RGSi Gai2 mutant mice show the expected antidepressant-like phenotype (reduced immobility) in the tail suspension test (TST) which is reversed by infusion of the selective 5-HT1AR antagonist WAY-100635 directly into the hippocampus. Conversely, direct hippocampal infusion of the 5-HT1AR agonist 8-OH-DPAT decreases immobility in wild type mice, and this is reversed by peripheral administration of WAY-100635. CCG-203769 is a small molecule RGS4 inhibitor with selectivity for RGS4 > RGS19 >> other RGS proteins. Intrahippocampal infusion of CCG-203769 over 7 days in wild-type mice produces a marked antidepressant-like activity in the TST. These findings identify hippocampal 5-HT1ARs specifically coupled to Gai2 as important for the antidepressant-like effect in this phenotype and suggest RGS4 and/or RGS19 as RGS proteins that may regulate signaling through this pathway. These results identify proteins along this signaling pathway (5-HT1AR/Gai2 complex, RGS4, RGS19) as potential targets for antidepressant medications.

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#### 784.07/K24. ***Behavioral effects of N,N-dialkyltryptamine hallucinogens in mice***

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N,N-dialkyltryptamines are known to act non-selectively as agonists at 5-HT1A and 5-HT2A receptors. However, the contributions of these receptors to the behavioral effects of N,N-dialkyltryptamines are unclear. Recently, illicit sources have made available an expanded repertoire of N,N-dialkyltryptamines, including N,N-dimethyltryptamine (DMT) and homologues with one or both methyl groups extended to ethyl or propyl groups. In light of increasing use of these compounds, we investigated their behavioral pharmacology in mice. The head twitch response (HTR), a behavior mediated by 5-HT2A, is often used as a rodent proxy for hallucinogenic effects in humans. Using a head-mounted magnet and a magnetometer coil to detect head movement, we found that IP treatment with DMT (0.625-10 mg/kg), N-methyl-N-ethyltryptamine (MET; 0.625-10 mg/kg), N,N-diethyltryptamine (DET; 0.3-3 mg/kg), and N,N-dipropyltryptamine (DPT; 0.625-10 mg/kg) induced the HTR in C57Bl/6J mice, suggesting that activity at 5-HT2A contributes to the behavioral effects of these compounds. Additionally, the Behavioral Pattern Monitor (BPM) was used to assess the effects of DMT, MET, DET, and DPT on exploratory behavior. When administered at 30 mg/kg IP, all four compounds reduced locomotor activity and investigatory behavior. Since previous BPM experiments demonstrated tryptamine hallucinogens to reduce locomotor activity by activating 5-HT1A (Halberstadt et al., 2011), we assessed the contribution of 5-HT1A to these locomotor effects by comparing the effects of DMT, MET, DET, and DPT in 5-HT1A wild-type (WT) and knockout (KO) mice. Interestingly, the involvement of 5-HT1A in locomotor hypoactivity depended on the length of the N-alkyl groups. The effects of DPT were completely absent in 5-HT1A KO mice, while the effects of DET were partially attenuated in the KOs. By contrast, DMT and MET produced similar responses in WT and KO mice, and the effect of DMT was not blocked by the 5-

HT1A antagonist WAY-100,635. Our findings demonstrate that the behavioral effects of N,N-dialkyltryptamines depend, at least in part, on the 5-HT<sub>2A</sub> receptor, and indicate a variable role for the 5-HT<sub>1A</sub> receptor depending on the length of the N-alkyl groups. Human clinical trials indicate subtle differences in the effects of DMT, DET, and DPT. In light of our findings, these subjective differences may result from differential interaction of these compounds with 5-HT<sub>1A</sub>. Experiments are in progress to determine the receptor(s) responsible for the effects of DMT and MET in the BPM.

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Location: Hall A  
Presentation time: Saturday, Oct 17, 2015, 1:00 PM - 5:00 PM  
Presenter at Poster: Sat, Oct. 17, 2015, 1:00 PM - 2:00 PM  
Topic: ++A.04.d. Axon growth and guidance: Extrinsic mechanisms

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**Abstract:** Engineered 3D microenvironments are enabling the creation of designer neuronal networks that approximate the geometry of neuronal tissues. Here we use 3D alginate hydrogels functionalized with RGD (Arginine-Glutamate-Aspartate) peptides to direct the growth of neuronal processes and shape the formation of the developing networks. Through a glacier-moraine process of freezing-drying poly-lactic-co-glycolic acid (PLGA) microparticles, random and uniaxially aligned porous microchannels were created within the gels (Lee et al., Adv. Healthc. Mat., 2015). We grew early postnatal neurons from rat hippocampal and also adult rat dorsal root ganglion (DRG) neurons in these gels. Neurite processes were significantly more directed on the uniaxially aligned hydrogels compared to those grown in the random-pored gels. Using immunofluorescence imaging, we found that the morphologies of the neuronal networks grown in the uniaxial gels from hippocampal and DRG neurons are very similar to laminar neurite alignments in the CA1 region of the hippocampus as well as in nerves extending from DRGs, respectively. To examine the functionality of the neuronal network, we used fluo-4 to monitor the intracellular Ca<sup>2+</sup> dynamics in the neurons. After stimulating the non-conductive gel locally using a tungsten microelectrode, we observed sharp Ca<sup>2+</sup> fluxes in the adjacent and downstream cells. This indicates that there was robust activation and communication between neurons within the hydrogel. Additionally, we measured the local field potential of the neuronal network within the gels and found activity characteristic of a communicating network. This study demonstrates the efficacy of porous 3D alginate hydrogels as scaffolds for developing neurons. Controlled growth of neurite processes using this scaffolding will be useful to better understand and direct cellular emergent behavior in differentiating and regenerating neurons.

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#### 5.02. *Epigenetic regulation of brain reward circuits*

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Department of Neurobiology, University of Alabama, Birmingham, AL.

Day JJ, Childs D, Guzman-Karlsson MC, Kibe M, Moulden J, Song E, Tahir A, Sweatt JD. DNA methylation regulates associative reward learning. *Nat Neurosci.* 16:1445-52, 2013.

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#### 5.03. *Stress-induced epigenetic changes in the FKBP5 locus.*

E. Binder;

Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA.

Provencal N, Binder EB. The neurobiological effects of stress as contributors to psychiatric disorders: focus on epigenetics. *Curr Opin Neurobiol.* 30C:31-37, 2014.

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#### 5.04. *Transcriptional and epigenetic bases of incubation of methamphetamine craving*

J. Cadet;

Molecular Neuropsychiatry Research Branch, NIH/NIDA Intramural Research Program, Baltimore, MD.

Krasnova IN, Marchant NJ, Ladenheim B, McCoy MT, Panlilio LV, Bossert JM, Shaham Y, Cadet JL.

Incubation of methamphetamine and palatable food craving after punishment-induced abstinence. *Neuropsychopharmacology.* 39:2008-16, 2014.

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#### 5.05. *Transcriptional and epigenetic effects of alcohol exposure*

K. Schuebel;

Laboratory of Neurogenetics, NIH/NIAAA Intramural Research Program, Bethesda.

Jeschke J, Van Neste L, Glöckner SC, Dhir M, Calmon MF, Deregowski V, Van Criekinge W, Vlassenbroeck I, Koch A, Chan TA, Cope L, Hooker CM, Schuebel KE, et al. Biomarkers for detection and prognosis of breast cancer identified by a functional hypermethylome screen. *Epigenetics.* 7:701-9, 2012

5.06. ***The impact of exposure of drugs of abuse on future generations***

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Vassoler FM, White SL, Schmidt HD, Sadri-Vakili G, Pierce RC. Epigenetic inheritance of a cocaine-resistance phenotype. *Nat Neurosci.* 16:42-7, 2013.

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5.07. ***Biological resilience to harsh and non-supporting parenting.***

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Department of Psychology, University of Pennsylvania, Philadelphia, PA.

Bowes L, Jaffee SR. Biology, genes, and resilience: toward a multidisciplinary approach. *Trauma Violence Abuse.* 14:195-208, 2013.

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114.05. ***Individual differences in avoidance learning correlate with dopamine-dependent function and neuro-circuitry***

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Reward and punishment learning plays a critical role in navigating a dynamic environment, and is thought to be impaired in several neuropsychiatric disorders including addiction. We currently have a limited understanding of individual differences in punishment processing and whether dopamine is critical to this function. Here, we combined an approach/avoidance learning task with cognitive assays of dopamine-dependent cognitive function and functional magnetic resonance imaging (fMRI) to investigate how dopaminergic function and circuitry modulate human avoidance behavior. Thirty-one healthy individuals participated in the study. Avoidance learning was tested using a probabilistic selection task (PS). Previous research indicates that performance on the Reading Span Task (RST) and Barratt Impulsiveness Scale (BIS) correlate with striatal dopamine synthesis and release, respectively. These cognitive measures were, therefore, collected from participants to indirectly assay diverse aspects of baseline dopamine function. To examine the underlying neural circuitry, fMRI was employed to measure BOLD activity during the PS task and resting state functional connectivity (rsFC) immediately preceding the task. Avoidance learning showed a significant interaction of RST and BIS, and suggested an inverted U-shaped effect of putative dopamine efficacy on avoidance learning. Concurrently, activity in bilateral dorsal striatum also revealed an RST - BIS interaction effect at feedback loss trials, with

activity in the right caudate correlating with avoidance performance. Finally, caudate-mediodorsal thalamus rsFC was influenced by the RST - BIS interaction, further bolstering the argument that dopaminergic mechanisms underlie the processing of loss and punishing stimuli. Taken together, our data suggests that a circuit consisting of the dorsal striatum together with its efferents may shape avoidance learning and may involve dopaminergic mechanisms. Our findings have implications for drug addiction and other neuropsychiatric disorders associated with punishment processing.

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